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<p>(54) Title: LARGE POROUS PARTICLES EMITTED FROM AN INHALER</p> <p>(57) Abstract</p> <p>Particles incorporating a surfactant and/or a hydrophilic or hydrophobic complex of a positively or negatively charged therapeutic agent and a charged molecule of opposite charge for drug delivery to the pulmonary system, and methods for their synthesis and administration are provided. In a preferred embodiment, the particles are made of a biodegradable material and have a tap density less than 0.4 g/cm³ and a mass mean diameter between 5 µm and 30 µm, which together yield an aerodynamic diameter of the particles of between approximately one and five microns. The particles may be formed of biodegradable materials such as biodegradable polymers. For example, the particles may be formed of poly(lactic acid) or poly(glycolic acid) or copolymers thereof. Alternatively, the particles may comprise a therapeutic, prophylactic or diagnostic agent and a material selected from the group consisting of surfactant and a molecule having a charge opposite to the charge of the agent and forming a complex thereto. The particles have a tap density less than 0.4 g/cm³ and a mass mean diameter between 5 µm and 30 µm. Exemplary surfactants include phosphoglycerides such as dipalmitoyl phosphatidylcholine (DPPC). The particles are administered to the respiratory tract to permit systemic or local delivery of a wide variety of therapeutic agents. Aggregation of particles before or during administration to the respiratory tract results in particles having an aerodynamic diameter larger than that of the fully dispersed particles. Aerodynamic diameters between three and five microns are advantageous for delivery to the central airways.</p>		

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LARGE POROUS PARTICLES EMITTED FROM AN INHALER

BACKGROUND OF THE INVENTION

The present application relates generally to particles for use in drug delivery to the pulmonary system.

5 Aerosols for the delivery of therapeutic agents to the respiratory tract have been described, for example, Adjei, A. and Garren, J. *Pharm. Res.*, 7: 565-569 (1990); and Zanen, P. and Lamm, J.-W.J. *Int. J. Pharm.*, 114: 111-115 (1995). The respiratory tract encompasses the upper airways, including the oropharynx and larynx, followed by the lower airways, which include the trachea followed by
10 bifurcations into the bronchi and bronchioli. The upper and lower airways are called the conducting airways. The terminal bronchioli then divide into respiratory bronchioli which then lead to the ultimate respiratory zone, the alveoli, or deep lung. Gonda, I. "Aerosols for delivery of therapeutic and diagnostic agents to the respiratory tract," in *Critical Reviews in Therapeutic Drug Carrier Systems*, 6: 273-
15 313 (1990). The deep lung, or alveoli, are the primary target of inhaled therapeutic aerosols for systemic drug delivery.

Inhaled aerosols have been used for the treatment of local lung disorders including asthma and cystic fibrosis (Anderson, *Am. Rev. Respir. Dis.*, 140: 1317-1324 (1989)) and have potential for the systemic delivery of peptides and proteins as
20 well (Patton and Platz, *Advanced Drug Delivery Reviews*, 8: 179-196 (1992)). However, pulmonary drug delivery strategies present many difficulties for the delivery of macromolecules; these include protein denaturation during aerosolization, excessive loss of inhaled drug in the oropharyngeal cavity (often exceeding 80%), poor control over the site of deposition, lack of reproducibility of
25 therapeutic results owing to variations in breathing patterns, the frequent too-rapid absorption of drug potentially resulting in local toxic effects, and phagocytosis by lung macrophages.

Considerable attention has been devoted to the design of therapeutic aerosol inhalers to improve the efficiency of inhalation therapies. Timsina *et. al.*, *Int. J. Pharm.*, 101: 1-13 (1995); and Tansey, I.P., *Spray Technol. Market*, 4: 26-29 (1994). Attention has also been given to the design of dry powder aerosol surface texture, regarding particularly the need to avoid particle aggregation, a phenomenon which considerably diminishes the efficiency of inhalation therapies. French, D.L., Edwards, D.A. and Niven, R.W., *J. Aerosol Sci.*, 27: 769-783 (1996). Dry powder formulations ("DPFs") with large particle size have improved flowability characteristics, such as less aggregation (Visser, J., *Powder Technology* 58: 1-10 (1989)), easier aerosolization, and potentially less phagocytosis. Rudt, S. and R.H. Muller, *J. Controlled Release*, 22: 263-272 (1992); Tabata, Y. and Y. Ikada, *J. Biomed. Mater. Res.*, 22: 837-858 (1988). Dry powder aerosols for inhalation therapy are generally produced with mean geometric diameters primarily in the range of less than 5 μ m. Ganderton, D., *J. Biopharmaceutical Sciences*, 3: 101-105 (1992); and Gonda, I. "Physico-Chemical Principles in Aerosol Delivery," in *Topics in Pharmaceutical Sciences 1991*, Crommelin, D.J. and K.K. Midha, Eds., Medpharm Scientific Publishers, Stuttgart, pp. 95-115, 1992. Large "carrier" particles (containing no drug) have been co-delivered with therapeutic aerosols to aid in achieving efficient aerosolization among other possible benefits. French, D.L., Edwards, D.A. and Niven, R.W., *J. Aerosol Sci.*, 27: 769-783 (1996).

The human lungs can remove or rapidly degrade hydrolytically cleavable deposited aerosols over periods ranging from minutes to hours. In the upper airways, ciliated epithelia contribute to the "mucociliary escalator" by which particles are swept from the airways toward the mouth. Pavia, D. "Lung Mucociliary Clearance," in *Aerosols and the Lung: Clinical and Experimental Aspects*, Clarke, S.W. and Pavia, D., Eds., Butterworths, London, 1984. Anderson, *Am. Rev. Respir. Dis.*, 140: 1317-1324 (1989). In the deep lungs, alveolar macrophages are capable of phagocytosing particles soon after their deposition. Warheit, M.B. and Hartsky, M.A., *Microscopy Res. Tech.*, 26: 412-422 (1993); Brain, J.D., "Physiology and Pathophysiology of Pulmonary Macrophages," in *The Reticuloendothelial System*, S.M. Reichard and J. Filkins, Eds., Plenum, New York, pp. 315-327, 1985; Dorries,

- A.M. and Valberg, P.A., *Am. Rev. Resp. Disease* 146: 831-837 (1991); and Gehr, P., *Microscopy Res. and Tech.*, 26: 423-436 (1993). As the diameter of particles exceeds 3 μm , there is increasingly less phagocytosis by macrophages. Kawaguchi, H., *Biomaterials* 7: 61-66 (1986); Krenis, L.J. and Strauss, B., *Proc. Soc. Exp. Med.*, 107: 748-750 (1961); and Rudt, S. and Muller, R.H., *J. Contr. Rel.*, 22: 263-272 (1992). However, increasing the particle size also has been found to minimize the probability of particles (possessing standard mass density) entering the airways and acini due to excessive deposition in the oropharyngeal or nasal regions. Heyder, J., *J. Aerosol Sci.*, 17: 811-825 (1986).
- 10 Local and systemic inhalation therapies can often benefit from a relatively slow controlled release of the therapeutic agent. Gonda, I., "Physico-chemical principles in aerosol delivery," in: *Topics in Pharmaceutical Sciences 1991*, D.J.A. Crommelin and K.K. Midha, Eds., Stuttgart: Medpharm Scientific Publishers, pp. 95-117 (1992). Slow release from a therapeutic aerosol can prolong the residence of
- 15 an administered drug in the airways or acini, and diminish the rate of drug appearance in the bloodstream. Also, patient compliance is increased by reducing the frequency of dosing. Langer, R., *Science*, 249: 1527-1533 (1990); and Gonda, I., "Aerosols for delivery of therapeutic and diagnostic agents to the respiratory tract," in *Critical Reviews in Therapeutic Drug Carrier Systems* 6: 273-313 (1990).
- 20 Controlled release drug delivery to the lung may simplify the way in which many drugs are taken. Gonda, I., *Adv. Drug Del. Rev.*, 5: 1-9 (1990); and Zeng, X., et al., *Int. J. Pharm.*, 124: 149-164 (1995). Pulmonary drug delivery is an attractive alternative to oral, transdermal, and parenteral administration because self-administration is simple, the lungs provide a large mucosal surface for drug
- 25 absorption, there is no first-pass liver effect of absorbed drugs, and there is reduced enzymatic activity and pH mediated drug degradation compared with the oral route. Relatively high bioavailability of many molecules, including macromolecules, can be achieved via inhalation. Wall, D.A., *Drug Delivery*, 2: 1-20 (1995); Patton, J. and Platz, R., *Adv. Drug Del. Rev.*, 8: 179-196 (1992); and Byron, P., *Adv. Drug Del. Rev.*, 5: 107-132 (1990). As a result, several aerosol formulations of therapeutic
- 30 drugs are in use or are being tested for delivery to the lung. Patton, J.S., et al., *J.*

Controlled Release, 28: 79-85 (1994); Damms, B. and Bains, W., *Nature Biotechnology* (1996); Niven, R.W., *et al.*, *Pharm. Res.*, 12(9): 1343-1349 (1995); and Kobayashi, S., *et al.*, *Pharm. Res.*, 13(1): 80-83 (1996).

Drugs currently administered by inhalation come primarily as liquid aerosol
5 formulations. However, many drugs and excipients, especially proteins, peptides
(Liu, R., *et al.*, *Biotechnol. Bioeng.*, 37: 177-184 (1991)), and biodegradable carriers
such as poly(lactide-co-glycolides) (PLGA), are unstable in aqueous environments
for extended periods of time. This can make storage as a liquid formulation
problematic. In addition, protein denaturation can occur during aerosolization with
10 liquid formulations. Mumenthaler, M., *et al.*, *Pharm. Res.*, 11: 12-20 (1994).
Considering these and other limitations, dry powder formulations (DPF's) are
gaining increased interest as aerosol formulations for pulmonary delivery. Damms,
B. and W. Bains, *Nature Biotechnology* (1996); Kobayashi, S., *et al.*, *Pharm. Res.*,
13(1): 80-83 (1996); and Timsina, M., *et al.*, *Int. J. Pharm.*, 101: 1-13 (1994).
15 However, among the disadvantages of DPF's is that powders of ultrafine particulates
usually have poor flowability and aerosolization properties, leading to relatively low
respirable fractions of aerosol, which are the fractions of inhaled aerosol that escape
deposition in the mouth and throat. Gonda, I., in *Topics in Pharmaceutical Sciences*
1991, D. Crommelin and K. Midha, Editors, Stuttgart: Medpharm Scientific
20 Publishers, 95-117 (1992). A primary concern with many aerosols is particulate
aggregation caused by particle-particle interactions, such as hydrophobic,
electrostatic, and capillary interactions. An effective dry-powder inhalation therapy
for both short and long term release of therapeutics, either for local or systemic
delivery, requires a powder that displays minimum aggregation, as well as a means
25 of avoiding or suspending the lung's natural clearance mechanisms until drugs have
been effectively delivered.

There is a need for improved inhaled aerosols for pulmonary delivery of
therapeutic agents. There is a need for the development of drug carriers which are
capable of delivering the drug in an effective amount into the airways or the alveolar
30 zone of the lung. There further is a need for the development of drug carriers for use
as inhaled aerosols which are biodegradable and are capable of controlled release of

-5-

drug within the airways or in the alveolar zone of the lung. There also is a need for particles for pulmonary drug delivery with improved aerosolization properties.

It is therefore an object of the present invention to provide improved carriers for the pulmonary delivery of therapeutic agents. It is a further object of the invention to provide inhaled aerosols which are effective carriers for delivery of therapeutic agents to the deep lung. It is another object of the invention to provide carriers for pulmonary delivery which avoid phagocytosis in the deep lung. It is a further object of the invention to provide carriers for pulmonary drug delivery which are capable of biodegrading and releasing the drug at a controlled rate. It is yet another object of the invention to provide particles for pulmonary drug delivery with improved aerosolization properties and optimized particle - particle interactions.

SUMMARY OF THE INVENTION

The invention is related to particles incorporating a surfactant and/or a hydrophilic or hydrophobic complex of a positively or negatively charged therapeutic agent and a charged molecule of opposite charge for delivery of therapeutic or diagnostic agents to the pulmonary system, and methods for their synthesis and administration, are provided. Exemplary surfactants include naturally occurring phosphatidylcholines, such as dipalmitoylphosphatidylcholine ("DPPC"). Exemplary hydrophilic or hydrophobic complexes include insulin (negatively charged) and protamine (positively charged). In a preferred embodiment, the particles are aerodynamically light particles, which are made of a biodegradable material, and have a tap density less than about 0.4 g/cm^3 . The "aerodynamically light" particles generally have a mean diameter between about $5 \text{ }\mu\text{m}$ and $30 \text{ }\mu\text{m}$, and an aerodynamic diameter resulting in delivery to the pulmonary system, including deep lung, central airways and upper airways. The tap density less than about 0.4 g/cm^3 and mean diameter between about $5 \text{ }\mu\text{m}$ and $30 \text{ }\mu\text{m}$, are designed to yield particles with an aerodynamic diameter between approximately one and five microns or, in some instances, more as desired. In another preferred embodiment, the particles have a mass density less than 0.4 g/cm^3 and a mean diameter between $5 \text{ }\mu\text{m}$ and $30 \text{ }\mu\text{m}$. The mass density, for example, generally less than 0.4 g/cm^3 and mean

diameter between 5 μm and 30 μm are designed to yield particles with an aerodynamic diameter between approximately one and five microns or more, or preferably, between one and three microns. The particles may be formed of biodegradable materials such as biodegradable polymers, proteins, surfactants or
5 other water soluble or non-water soluble materials. Particles can also be formed of water-soluble excipients, such as trehalose or lactose, or proteins, such as the proteins to be delivered. In one embodiment, the particles include only a therapeutic, prophylactic or diagnostic agent to be delivered to a patient in a complex with another charged molecule. In a second embodiment, the particles include only
10 the agent and a surfactant. In a third embodiment, particles include surfactant and charged molecules forming a complex, which can provide a sustained release.

The particles can be used for enhanced delivery of a therapeutic agent to the airways or the alveolar region of the lung. The particles may be effectively aerosolized for administration to the respiratory tract to permit systemic or local
15 delivery of a wide variety of therapeutic agents. They also optionally may be co-delivered with larger carrier particles, not carrying a therapeutic agent, having, for example, a mean diameter ranging between about 50 μm and 100 μm . The particles can be used to form a composition that includes the particles and a pharmaceutically acceptable carrier for administration to a patient, preferably for administration via
20 inhalation.

According to one embodiment of the invention, aerodynamically light particles can themselves be used as carriers for the delivery of a therapeutic, prophylactic or diagnostic agent to the pulmonary system. Such aerodynamically light carriers can include one or more surfactants, biocompatible polymers and/or
25 excipients such as those further described below. According to this embodiment of the invention, a therapeutic, prophylactic or diagnostic agent can be added onto the aerodynamically light carrier for delivery to the pulmonary system. Small-sized therapeutic, prophylactic or diagnostic agents, such as, for example, agents having a particle size in the nanometer range, can be carried by the aerodynamically light
30 carriers and delivered to the pulmonary system.

The invention is also related to a method for drug delivery to the pulmonary system employing the particles described herein. The method comprises administering to the respiratory tract of a patient in need of treatment, prophylaxis or diagnosis an effective amount of particles comprising a therapeutic, prophylactic or diagnostic agent and a material selected from the group consisting of a surfactant and a molecule having a charge opposite to the charge of the therapeutic agent and forming a complex thereto. According to one embodiment of the invention, the particles have a tap density less than about 0.4 g/cm^3 and a mean diameter of between about $5 \text{ }\mu\text{m}$ and $30 \text{ }\mu\text{m}$. According to another embodiment of the invention, the particles have a mass density less than about 0.4 g/cm^3 and a mean diameter of between about $5 \text{ }\mu\text{m}$ and $30 \text{ }\mu\text{m}$.

In one embodiment, the method of the invention is related to administering to the respiratory tract of a patient particles having an aerodynamic diameter between approximately one and five microns. In a preferred embodiment, the method of the invention is related to administering to the respiratory tract of a patient particles having an aerodynamic diameter between approximately one and three microns. In another preferred embodiment, the method of the invention is related to administering to the respiratory tract of a patient particles having an aerodynamic diameter between approximately three and five microns.

In one embodiment, the method for drug delivery to the pulmonary system comprises administering to the respiratory tract of a patient in need of treatment, prophylaxis or diagnosis an effective amount of particles comprising a therapeutic, diagnostic or prophylactic agent and a molecule selected from the group consisting of a surfactant and a molecule having a charge opposite to the charge of the therapeutic agent and forming a complex thereto, wherein the particles have a tap density less than about 0.4 g/cm^3 and a mean diameter of between about $5 \text{ }\mu\text{m}$ and $30 \text{ }\mu\text{m}$, effective to yield an aerodynamic diameter of the particles of between approximately one and three microns and wherein, before or during administering to the respiratory tract, the particles are aggregated to form aggregated particles. The aggregated particles have an aerodynamic diameter between approximately three and five microns. As used herein, the term "aggregated", "aggregate" or "aggregation" is

interchangeable in meaning with the term "agglomerated", "agglomerate" or "agglomeration".

According to one embodiment of the invention, the particles are non-polymeric as further defined below.

- 5 The invention has numerous advantages. For example, the invention provides a method whereby the aerodynamic diameter of a given lot of particles, having a defined mean diameter and tap density, may be tuned to meet the requirements of specific regions of the pulmonary systems.

BRIEF DESCRIPTION OF THE DRAWINGS

- 10 Figure 1 is a graph comparing the mass fraction of the initial dose that is released from a dry powder inhaler device, after *in vitro* aerosolization of poly (D,L-lactic-co-glycolic acid) ("PLGA") microspheres made by a double emulsion procedure with and without the incorporation of L- α -phosphatidylcholine dipalmitoyl ("DPPC").

- 15 Figure 2 is a graph comparing the mass fraction of the aerosolized dose that is deposited in different stages of a cascade impactor after *in vitro* aerosolization of PLGA microspheres made by a double emulsion procedure with and without the incorporation of DPPC.

- 20 Figure 3 is a graph showing the aerosolization behavior of PLGA microspheres made by spray drying with and without the incorporation of DPPC showing the mass-fraction of the initial dose that is released from the dry powder inhaler device after *in vitro* aerosolization.

- 25 Figure 4 is a graph comparing the *in vitro* aerosolization behaviors of PLA and PLGA microspheres made by spray drying with and without the incorporation of DPPC showing the mass-fraction of the aerosolized dose that is deposited in stages of a cascade impactor corresponding to the "respirable-fraction".

Figure 5 is a graph comparing the plasma concentration of insulin (ng/ml) per unit time (hrs).

Figure 6 is a graph comparing the release of albuterol (%) over time (hrs).

Figure 7 is a graph comparing the *in vitro* release of albuterol (%) over time (hrs) for compositions with varying ratios of DPPC, albumin, lactose and albuterol.

Figure 8 is a graph comparing the airway resistance change (cm H₂O/ml/sec.) per unit time (hrs).

5 Figure 9 is a plot comparing the aerodynamic diameter of fully dispersed particles with the aerodynamic diameter of aggregated particles.

Figure 10 is a plot showing the average aerodynamic diameter of particles measured by a time of flight device (API Aerosizer^(R) and Aerodisperser^(R)) as a function of shear rate.

10 Figure 11 is a plot showing the aerodynamic size distribution following exit from a dry powder inhaler.

DETAILED DESCRIPTION OF THE INVENTION

The features and other details of the invention, either as steps of the invention or as combination of parts of the invention, will now be more particularly
15 described with reference to the accompanying drawings and pointed out in the claims. The same numeral present in different figures represents the same item. It will be understood that the particular embodiments of the invention are shown by way of illustration and not as limitations of the invention. The principle feature of this invention may be employed in various embodiments without departing from the
20 scope of the invention.

The invention is related to particles incorporating a surfactant and/or a hydrophilic or hydrophobic complex of a positively or negatively charged therapeutic, prophylactic or diagnostic agent and a charged molecule of opposite charge for delivery to the pulmonary system, and methods for their synthesis and
25 administration are provided. The particles can, but need not include a therapeutic, prophylactic or diagnostic agent. In one embodiment, the particles include either only a therapeutic, prophylactic or diagnostic agent for delivery to a patient. In a second embodiment, the particles include a therapeutic, prophylactic or diagnostic agent and a surfactant.

The particles have a tap density less than 0.4 g/cm^3 and a mean diameter between $5 \text{ }\mu\text{m}$ and $30 \text{ }\mu\text{m}$, which in combination yield an aerodynamic diameter of between one and five microns, preferably between one and three microns. The aerodynamic diameter is calculated to provide for maximum deposition within the lungs, previously achieved by the use of very small particles of less than five microns in diameter, preferably between one and three microns, which are then subject to phagocytosis. Selection of particles which have a larger diameter, but which are sufficiently light (hence the characterization "aerodynamically light"), results in an equivalent delivery to the lungs, but the larger size particles are not phagocytosed. Improved delivery can be obtained by using particles with a rough or uneven surface relative to those with a smooth surface. Generally, the presence of a surfactant minimizes undesirable aggregation of the particles. However, as discussed in more detail below, the invention is further related to a method of delivery to the pulmonary system wherein some aggregation of particles is used to obtain particles having an aerodynamic diameter larger than that of the fully dispersed particles. The presence of a complex of the therapeutic agent with a molecule of opposite charge provides for sustained release of the agent.

According to one embodiment of the invention, the particles have a mass density of less than about 0.4 g/cm^3 and a mean diameter of between $5 \text{ }\mu\text{m}$ and $30 \text{ }\mu\text{m}$. Mass density and the relationship between mass density, mean diameter and aerodynamic diameter are discussed in U. S. Application No. 08/655,570, filed on May 24, 1996, which is incorporated herein by reference in its entirety. In a preferred embodiment, the aerodynamic diameter of particles having a mass density less than about 0.4 g/cm^3 and a mean diameter of between about $5 \text{ }\mu\text{m}$ and $30 \text{ }\mu\text{m}$ is between one and five microns.

The particles can be used for controlled systemic or local delivery of therapeutic or diagnostic agents to the respiratory tract via aerosolization. Administration of the particles to the lung by aerosolization permits deep lung delivery of relatively large diameter therapeutic aerosols, for example, greater than $5 \text{ }\mu\text{m}$ in mean diameter. The particles can be fabricated with a rough surface texture to reduce particle agglomeration and improve flowability of the powder. The particles

have improved aerosolization properties. The particle can be fabricated with features which enhance aerosolization via dry powder inhaler devices, and lead to lower deposition in the mouth, throat and inhaler device.

- 5 The particles can be used to form a composition that includes the particles and a pharmaceutically acceptable carrier for administration to a patient, preferably for administration via inhalation. Suitable carriers include those typically used for inhalation therapy. Those of skill in the art can readily determine an appropriate pharmaceutically acceptable carrier for use in administering particles via inhalation.

Particle Materials

- 10 The particles can be prepared entirely from a therapeutic or diagnostic agent, or from a combination of the agent and a surfactant. The particles preferably are biodegradable and biocompatible, and optionally are capable of biodegrading at a controlled rate for delivery of a therapeutic or diagnostic agent. The particles can be made of a variety of materials. Both inorganic and organic materials can be used.
- 15 For example, ceramics may be used. Polymeric and non-polymeric materials, such as fatty acids, may be used to form aerodynamically light particles. Other suitable materials include, but are not limited to, gelatin, polyethylene glycol, trehalose, and dextran. Particles with degradation and release times ranging from seconds to months can be designed and fabricated, based on factors such as the particle
- 20 material. Different properties of the particle which can contribute to the aerodynamic lightness include the composition forming the particle, and the presence of irregular surface structure, or pores or cavities within the particle.

Polymeric Particles

- 25 Polymeric particles may be formed from any biocompatible, and preferably biodegradable polymer, copolymer, or blend. Preferred polymers are those which are capable of forming aerodynamically light particles having a tap density less than

about 0.4 g/cm³, a mean diameter between 5 μm and 30 μm and an aerodynamic diameter between approximately one and five microns, preferably between one and three microns. The polymers may be tailored to optimize different characteristics of the particle including: i) interactions between the agent to be delivered and the
5 polymer to provide stabilization of the agent and retention of activity upon delivery; ii) rate of polymer degradation and, thereby, rate of drug release profiles; iii) surface characteristics and targeting capabilities via chemical modification; and iv) particle porosity.

Surface eroding polymers such as polyanhydrides may be used to form the
10 particles. For example, polyanhydrides such as poly[(*p*-carboxyphenoxy)-hexane anhydride] (PCPH) may be used. Biodegradable polyanhydrides are described in U.S. Patent No. 4,857,311.

In another embodiment, bulk eroding polymers such as those based on polyesters including poly(hydroxy acids) can be used. For example, polyglycolic
15 acid (PGA), polylactic acid (PLA), or copolymers thereof may be used to form the particles. The polyester may also have a charged or functionalizable group, such as an amino acid. In a preferred embodiment, particles with controlled release properties can be formed of poly(D,L-lactic acid) and/or poly(D,L-lactic-co-glycolic acid) ("PLGA") which incorporate a surfactant such as DPPC.

20 Other polymers include polyamides, polycarbonates, polyalkylenes such as polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly vinyl compounds such as polyvinyl alcohols, polyvinyl ethers, and polyvinyl esters, polymers of acrylic and methacrylic acids, celluloses and other polysaccharides, and peptides or proteins, or copolymers or
25 blends thereof. Polymers may be selected with or modified to have the appropriate stability and degradation rates *in vivo* for different controlled drug delivery applications.

In one embodiment, aerodynamically light particles are formed from functionalized polyester graft copolymers, as described in Hrkach *et al.*,
30 *Macromolecules*, 28: 4736-4739 (1995); and Hrkach *et al.*, "Poly(L-Lactic acid-co-amino acid) Graft Copolymers: A Class of Functional Biodegradable Biomaterials"

in *Hydrogels and Biodegradable Polymers for Bioapplications*, ACS Symposium Series No. 627, Raphael M. Ottenbrite *et al.*, Eds., American Chemical Society, Chapter 8, pp. 93-101, 1996.

Materials other than biodegradable polymers may be used to form the
5 particles. Suitable materials include various non-biodegradable polymers and various excipients.

The particles also may be formed of a therapeutic or diagnostic agent and surfactant alone. In one embodiment, the particles may be formed of the surfactant and include a therapeutic or diagnostic agent, to improve aerosolization efficiency
10 due to reduced particle surface interactions, and to potentially reduce loss of the agent due to phagocytosis by alveolar macrophages.

Non-Polymeric particles

As used herein, the term "non-polymeric particles" refers to particles that do not include polymers such as the polymers described in the section above. To
15 provide one specific example, non-polymeric particles do not include PLA, PGA or PLGA.

In one embodiment, non-polymeric particles comprise a therapeutic, prophylactic or diagnostic agent and a surfactant. In another embodiment, non-polymeric particles comprise a therapeutic, prophylactic or diagnostic agent and a
20 molecule having a charge opposite to the charge of the agent and forming a complex thereto. According to one embodiment of the invention, non-polymeric particles have a tap density less than 0.4 g/cm³ and a mean diameter between 5 µm and 30 µm. According to another embodiment of the invention, non-polymeric particles have a mass density less than 0.4 g/cm³ and a mean diameter between 5 µm and 30
25 µm.

Non-polymeric particles can further include excipients, such as described below.

Excipients

In addition to a therapeutic or diagnostic agent (or possibly other desired molecules for delivery), the particles can include, and preferably, do include, one or more of the following excipients; a sugar, such as lactose, a protein, such as
5 albumin, and/or a surfactant.

Complex Forming Materials

If the agent to be delivered is negatively charged (such as insulin), protamine or other positively charged molecules can be added to provide a lipophilic complex which results in the sustained release of the negatively charged agent. Negatively
10 charged molecules can be used to render insoluble positively charged agents.

Surfactants

Surfactants which can be incorporated into particles to improve their aerosolization properties include phosphoglycerides. Exemplary phosphoglycerides include phosphatidylcholines, such as the naturally occurring surfactant,
15 L- α -phosphatidylcholine dipalmitoyl ("DPPC"). The surfactants advantageously improve surface properties by, for example, reducing particle-particle interactions, and can render the surface of the particles less adhesive. The use of surfactants endogenous to the lung may avoid the need for the use of non-physiologic surfactants.

20 As used herein, the term "surfactant" refers to any agent which preferentially absorbs to an interface between two immiscible phases, such as the interface between water and an organic polymer solution, a water/air interface or organic solvent/air interface. Surfactants generally possess a hydrophilic moiety and a lipophilic moiety, such that, upon absorbing to microparticles, they tend to present
25 moieties to the external environment that do not attract similarly-coated particles, thus reducing particle agglomeration. Surfactants may also promote absorption of a therapeutic or diagnostic agent and increase bioavailability of the agent.

As used herein, a particle "incorporating a surfactant" refers to a particle with a surfactant on at least the surface of the particle. The surfactant may be

incorporated throughout the particle and on the surface during particle formation, or may be coated on the particle after particle formation. The surfactant can be coated on the particle surface by adsorption, ionic or covalent attachment, or physically "entrapped" by the surrounding matrix. The surfactant can be, for example,
5 incorporated into controlled release particles, such as polymeric microspheres.

Providing a surfactant on the surfaces of the particles can reduce the tendency of the particles to agglomerate due to interactions such as electrostatic interactions, Van der Waals forces, and capillary action. The presence of the surfactant on the particle surface can provide increased surface rugosity (roughness),
10 thereby improving aerosolization by reducing the surface area available for intimate particle-particle interaction. The use of a surfactant which is a natural material of the lung can potentially reduce opsonization (and thereby reducing phagocytosis by alveolar macrophages), thus providing a longer-lived controlled release particle in the lung.

15 Surfactants known in the art can be used including any naturally occurring surfactant. Other exemplary surfactants include diphosphatidyl glycerol (DPPG); hexadecanol; fatty alcohols such as polyethylene glycol (PEG); polyoxyethylene-9-lauryl ether; a surface active fatty acid, such as palmitic acid or oleic acid; sorbitan trioleate (Span 85); glycocholate; surfactin; a poloxomer; a sorbitan fatty acid ester
20 such as sorbitan trioleate; tyloxapol and a phospholipid.

Materials Enhancing Sustained Release

If the molecules are hydrophilic and tend to solubilize readily in an aqueous environment, another method for achieving sustained release is to use cholesterol or very high surfactant concentration. This complexation methodology also applies to
25 particles that are not aerodynamically light.

Formation of Particles

Formation of Polymeric Particles

Polymeric particles may be prepared using single and double emulsion solvent evaporation, spray drying, solvent extraction, solvent evaporation, phase separation, simple and complex coacervation, interfacial polymerization, supercritical carbon dioxide (CO₂) and other methods well known to those of ordinary skill in the art. Particles may be made using methods for making microspheres or microcapsules known in the art, provided that the conditions are optimized for forming particles with the desired aerodynamic diameter, or additional steps are performed to select particles with the density and diameter sufficient to provide the particles with an aerodynamic diameter between one and five microns, preferably between one and three microns.

Methods developed for making microspheres for delivery of encapsulated agents are described in the literature, for example, as described in Doubrow, M., Ed., "Microcapsules and Nanoparticles in Medicine and Pharmacy," CRC Press, Boca Raton, 1992. Methods also are described in Mathiowitz and Langer, *J. Controlled Release* 5: 13-22 (1987); Mathiowitz, *et al.*, *Reactive Polymers* 6: 275-283 (1987); and Mathiowitz *et al.*, *J. Appl. Polymer Sci.* 35: 755-774 (1988). The selection of the method depends on the polymer selection, the size, external morphology, and crystallinity that is desired, as described, for example, by Mathiowitz, *et al.*, *Scanning Microscopy* 4: 329-340 (1990); Mathiowitz, *et al.*, *J. Appl. Polymer Sci.* 45: 125-134 (1992); and Benita, *et al.*, *J. Pharm. Sci.* 73: 1721-1724 (1984).

In solvent evaporation, described for example, in Mathiowitz, *et al.*, (1990), Benita; and U.S. Patent No. 4,272,398 to Jaffe, the polymer is dissolved in a volatile organic solvent, such as methylene chloride. Several different polymer concentrations can be used, for example, between 0.05 and 1.0 g/ml. The therapeutic or diagnostic agent, either in soluble form or dispersed as fine particles, is added to the polymer solution, and the mixture is suspended in an aqueous phase that contains a surface active agent such as poly(vinyl alcohol). The aqueous phase may be, for example, a concentration of 1% poly(vinyl alcohol) w/v in distilled

water. The resulting emulsion is stirred until most of the organic solvent evaporates, leaving solid microspheres, which may be washed with water and dried overnight in a lyophilizer. Microspheres with different sizes (between 1 and 1000 microns) and morphologies can be obtained by this method.

5 Solvent removal was primarily designed for use with less stable polymers, such as the polyanhydrides. In this method, the agent is dispersed or dissolved in a solution of a selected polymer in a volatile organic solvent like methylene chloride. The mixture is then suspended in oil, such as silicon oil, by stirring, to form an emulsion. Within 24 hours, the solvent diffuses into the oil phase and the emulsion
10 droplets harden into solid polymer microspheres. Unlike the hot-melt microencapsulation method described for example in Mathiowitz, *et al.*, *Reactive Polymers*, 6: 275 (1987), this method can be used to make microspheres from polymers with high melting points and a wide range of molecular weights. Microspheres having a diameter for example between one and 300 microns can be
15 obtained with this procedure.

With some polymeric systems, polymeric particles prepared using a single or double emulsion technique vary in size depending on the size of the droplets. If droplets in water-in-oil emulsions are not of a suitably small size to form particles with the desired size range, smaller droplets can be prepared, for example, by
20 sonication or homogenization of the emulsion, or by the addition of surfactants.

If the particles prepared by any of the above methods have a size range outside of the desired range, particles can be sized, for example, using a sieve, and further separated according to density using techniques known to those of skill in the art. Alternatively, particles larger than the size range can be milled or ground, while
25 those less than the size range need only be emitted.

The polymeric particles are preferably prepared by spray drying. Prior methods of spray drying, such as that disclosed in PCT WO 96/09814 by Sutton and Johnson, disclose the preparation of smooth, spherical microparticles of a water-soluble material with at least 90% of the particles possessing a mean size between 1
30 and 10 μm . The method disclosed herein provides rough (non-smooth), non-spherical microparticles that include a water-soluble material combined with a

water-insoluble material. At least 90% of the particles possess a mean size between 5 and 30 μm , and a low mass or tap density (less than 0.4 g/cm^3).

The particles can incorporate various complexes of therapeutic or diagnostic agents to be delivered with molecules of an opposite charge, or can include
5 substances such as lipids which allow for the sustained release of small and large molecules. Addition of these complexes or substances is applicable to particles of any size and shape, and is especially useful for altering the rate of release of therapeutic agents from inhaled particles.

Aerodynamically Light Particles

10 Aerodynamically light particles of the invention, may be fabricated using the methods disclosed herein.

Aerodynamically Light Particle Size

The mass mean diameter of the particles can be measured using an electrical zone sensing instrument such as Coulter Multisizer IIe (Coulter Electronics, Luton,
15 Beds, England) or a laser diffraction instrument (for example Helos, Sympatec, New Jersey).. The aerodynamically light particles in one preferred embodiment are at least about 5 microns in diameter. The diameter of particles in a sample will range depending upon factors such as particle composition and methods of synthesis. The distribution of size of particles in a sample can be selected to permit optimal
20 deposition within targeted sites within the respiratory tract.

The aerodynamically light particles may be fabricated or separated, for example by filtration or centrifugation, to provide a particle sample with a preselected size distribution. For example, greater than 30%, 50%, 70%, or 80% of the particles in a sample can have a diameter within a selected range of at least 5
25 μm . The selected range within which a certain percentage of the particles must fall may be for example, between about 5 and 30 μm , or optionally between 5 and 15 μm . In one preferred embodiment, at least a portion of the particles have a diameter between about 9 and 11 μm . Optionally, the particle sample also can be fabricated wherein at least 90%, or optionally 95% or 99%, have a diameter within the selected

range. The presence of the higher proportion of the aerodynamically light, larger diameter (at least about 5 μm) particles in the particle sample enhances the delivery of therapeutic or diagnostic agents incorporated therein to the deep lung.

In one embodiment, in the particle sample, the interquartile range may be 2 μm , with a mean diameter for example, between about 7.5 and 13.5 μm . Thus, for example, between at least 30% and 40% of the particles may have diameters within the selected range. Preferably, the said percentages of particles have diameters within a 1 μm range, for example, between 6.0 and 7.0 μm , 10.0 and 11.0 μm or 13.0 and 14.0 μm .

The aerodynamically light particles, optionally incorporating a therapeutic or diagnostic agent, with a tap density less than about 0.4 g/cm^3 , mean diameters of at least about 5 μm , and an aerodynamic diameter of between one and five microns, preferably between one and three microns, are more capable of escaping inertial and gravitational deposition in the oropharyngeal region, and are targeted to the airways or the deep lung. The use of larger particles (mean diameter at least about 5 μm) is advantageous since they are able to aerosolize more efficiently than smaller, denser aerosol particles such as those currently used for inhalation therapies.

In comparison to smaller, relatively denser particles, the larger (at least about 5 μm) aerodynamically light particles also can potentially more successfully avoid phagocytic engulfment by alveolar macrophages and clearance from the lungs, due to size exclusion of the particles from the phagocytes' cytosolic space. Phagocytosis of particles by alveolar macrophages diminishes precipitously as particle diameter increases beyond 3 μm . Kawaguchi, H., *et al.*, *Biomaterials* 7: 61-66 (1986); Krenis, L.J. and Strauss, B., *Proc. Soc. Exp. Med.*, 107: 748-750 (1961); and Rudt, S. and Muller, R.H., *J. Contr. Rel.*, 22: 263-272 (1992). For particles of statistically isotropic shape, such as spheres with rough surfaces, the particle envelope volume is approximately equivalent to the volume of cytosolic space required within a macrophage for complete particle phagocytosis.

Aerodynamically light particles thus are capable of a longer term release of an encapsulated agent in the lungs. Following inhalation, aerodynamically light biodegradable particles can deposit in the lungs (due to their relatively low tap

density), and subsequently undergo slow degradation and drug release, without the majority of the particles being phagocytosed by alveolar macrophages. The drug can be delivered relatively slowly into the alveolar fluid, and at a controlled rate into the blood stream, minimizing possible toxic responses of exposed cells to an excessively high concentration of the drug. The aerodynamically light particles thus are highly suitable for inhalation therapies, particularly in controlled release applications.

The preferred mean diameter for aerodynamically light particles for inhalation therapy is at least about 5 μm , for example between about 5 and 30 μm . The particles may be fabricated with the appropriate material, surface roughness, diameter and tap density for localized delivery to selected regions of the respiratory tract such as the deep lung or upper airways. For example, higher density or larger particles may be used for upper airway delivery, or a mixture of different sized particles in a sample, provided with the same or different therapeutic agent may be administered to target different regions of the lung in one administration.

15 Density and Deposition of Aerodynamically Light Particles

As used herein, the phrase "aerodynamically light particles" refers to particles having a tap density less than about 0.4 g/cm³. The tap density of particles of a dry powder may be obtained using a GeoPycTM instrument (Micrometrics Instrument Corp., Norcross, GA 30093). A Dual Platform Microprocessor Controlled Tap Density Tester (Vankel, NC) can also be used. Tap density is a standard measure of the envelope mass density. The envelope mass density of an isotropic particle is defined as the mass of the particle divided by the minimum sphere envelope volume within which it can be enclosed. Features which can contribute to low tap density include irregular surface texture and porous structure.

25 Inertial impaction and gravitational settling of aerosols are predominant deposition mechanisms in the airways and acini of the lungs during normal breathing conditions. Edwards, D.A., *J. Aerosol Sci.*, 26: 293-317 (1995). The importance of both deposition mechanisms increases in proportion to the mass of aerosols and not to particle (or envelope) volume. Since the site of aerosol deposition in the lungs is determined by the mass of the aerosol (at least for particles of mean aerodynamic

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diameter greater than approximately 1 μm), diminishing the tap density by increasing particle surface irregularities and particle porosity permits the delivery of larger particle envelope volumes into the lungs, all other physical parameters being equal.

The low tap density particles have a small aerodynamic diameter in
 5 comparison to the actual envelope sphere diameter. The aerodynamic diameter, d_{aer} , is related to the envelope sphere diameter, d (Gonda, I., "Physico-chemical principles in aerosol delivery," in *Topics in Pharmaceutical Sciences 1991* (eds. D.J.A. Crommelin and K.K. Midha), pp. 95-117, Stuttgart: Medpharm Scientific Publishers, 1992)), by the formula:

$$10 \quad d_{\text{aer}} = d\sqrt{\rho}$$

where the envelope mass ρ is in units of g/cm^3 . Maximal deposition of monodisperse aerosol particles in the alveolar region of the human lung (~60%) occurs for an aerodynamic diameter of approximately $d_{\text{aer}}=3 \mu\text{m}$. Heyder, J. *et al.*, *J. Aerosol Sci.*, 17: 811-825 (1986). Due to their small envelope mass density, the
 15 actual diameter d of aerodynamically light particles comprising a monodisperse inhaled powder that will

exhibit maximum deep-lung deposition is:

$$d = 3/\sqrt{\rho} \mu\text{m} \text{ (where } \rho < 1 \text{ g}/\text{cm}^3\text{);}$$

20 where d is always greater than 3 μm . For example, aerodynamically light particles that display an envelope mass density, $\rho = 0.1 \text{ g}/\text{cm}^3$, will exhibit a maximum deposition for particles having envelope diameters as large as 9.5 μm . The increased particle size diminishes interparticle adhesion forces. Visser, J., *Powder Technology*, 58: 1-10. Thus, large particle size increases efficiency of aerosolization

to the deep lung for particles of low envelope mass density, in addition to contributing to lower phagocytic losses.

Targeting of Particles

Targeting molecules can be attached to the particles via reactive functional groups on the particles. For example, targeting molecules can be attached to the amino acid groups of functionalized polyester graft copolymer particles, such as poly(lactic acid-co-lysine) (PLAL-Lys) particles. Targeting molecules permit binding interaction of the particle with specific receptor sites, such as those within the lungs. The particles can be targeted by attachment of ligands which specifically or non-specifically bind to particular targets. Exemplary targeting molecules include antibodies and fragments thereof including the variable regions, lectins, and hormones or other organic molecules capable of specific binding, for example, to receptors on the surfaces of the target cells.

Therapeutic or Prophylactic Agents

Any of a variety of therapeutic or prophylactic agents can be incorporated within the particles, or used to prepare particles consisting solely of the agent and surfactant. The particles can be used to locally or systemically deliver a variety of incorporated agents to an animal. Examples include synthetic inorganic and organic compounds, proteins and peptides, polysaccharides and other sugars, lipids, and DNA and RNA nucleic acid sequences having therapeutic, prophylactic or diagnostic activities. Nucleic acid sequences include genes, antisense molecules which bind to complementary DNA to inhibit transcription, and ribozymes. The agents to be incorporated can have a variety of biological activities, such as vasoactive agents, neuroactive agents, hormones, anticoagulants, immunomodulating agents, cytotoxic agents, prophylactic agents, antibiotics, antivirals, antisense, antigens, and antibodies. In some instances, the proteins may be antibodies or antigens which otherwise would have to be administered by injection to elicit an appropriate response. Compounds with a wide range of molecular weight can be encapsulated, for example, between 100 and 500,000 grams or more per mole.

Proteins are defined as consisting of 100 amino acid residues or more; peptides are less than 100 amino acid residues. Unless otherwise stated, the term protein refers to both proteins and peptides. Examples include insulin and other hormones. Polysaccharides, such as heparin, can also be administered.

5 The polymeric aerosols are useful as carriers for a variety of inhalation therapies. They can be used to encapsulate small and large drugs, release encapsulated drugs over time periods ranging from hours to months, and withstand extreme conditions during aerosolization or following deposition in the lungs that might otherwise harm the encapsulated therapeutic.

10 The particles may include a therapeutic agent for local delivery within the lung, such as agents for the treatment of asthma, emphysema, or cystic fibrosis, or for systemic treatment. For example, genes for the treatment of diseases such as cystic fibrosis can be administered, as can beta agonists for asthma. Other specific therapeutic agents include, but are not limited to, human growth hormone, insulin, 15 calcitonin, leuprolide (or gonadotropin-releasing hormone ("LHRH")), granulocyte colony-stimulating factor ("G-CSF"), parathyroid hormone-related peptide, somatostatin, testosterone, progesterone, estradiol, nicotine, fentanyl, norethisterone, clonidine, scopolamine, salicylate, cromolyn sodium, salmeterol, formeterol, albuterol, and valium.

20 Those therapeutic agents which are charged, such as most of the proteins, including insulin, can be administered as a complex between the charged therapeutic agent and a molecule of opposite charge. Preferably, the molecule of opposite charge is a charged lipid or an oppositely charged protein.

Diagnostic agents

25 Any of a variety of diagnostic agents can be incorporated within the particles, which can locally or systemically deliver the incorporated agents following administration to a patient. Any biocompatible or pharmacologically acceptable gas can be incorporated into the particles or trapped in the pores of the particles using technology known to those skilled in the art. The term gas refers to any compound 30 which is a gas or capable of forming a gas at the temperature at which imaging is

being performed. In one embodiment, retention of gas in the particles is improved by forming a gas-impermeable barrier around the particles. Such barriers are well known to those of skill in the art.

Other imaging agents which may be utilized include commercially available
5 agents used in positron emission tomography (PET), computer assisted tomography (CAT), single photon emission computerized tomography, x-ray, fluoroscopy, and magnetic resonance imaging (MRI).

Examples of suitable materials for use as contrast agents in MRI include the gadolinium chelates currently available, such as diethylene triamine pentacetic acid
10 (DTPA) and gadopentotate dimeglumine, as well as iron, magnesium, manganese, copper and chromium.

Examples of materials useful for CAT and x-rays include iodine based materials for intravenous administration, such as ionic monomers typified by diatrizoate and iothalamate, non-ionic monomers such as iopamidol, isohexol, and
15 ioversol, non-ionic dimers, such as iotrol and iodixanol, and ionic dimers, for example, ioxagalte.

Porous particles can be prepared which can be delivered via pulmonary delivery, and used, for example, for local or systemic delivery of incorporated agents and/or for imaging purposes. Particles incorporating diagnostic agents can be
20 detected using standard techniques available in the art and commercially available equipment.

Administration

The particles may be administered alone or in any appropriate pharmaceutically acceptable carrier, such as a liquid, for example saline, or a
25 powder, for administration to the respiratory system. They can be co-delivered with larger carrier particles, not including a therapeutic agent, the latter possessing mass mean diameters for example in the range between 50 μm and 100 μm .

Aerosol dosage, formulations and delivery systems may be selected for a particular therapeutic application, as described, for example, in Gonda, I. "Aerosols
30 for delivery of therapeutic and diagnostic agents to the respiratory tract," in *Critical*

Reviews in Therapeutic Drug Carrier Systems, 6: 273-313, 1990; and in Moren, "Aerosol dosage forms and formulations," in: *Aerosols in Medicine. Principles, Diagnosis and Therapy*, Moren, *et al.*, Eds, Eisevier, Amsterdam, 1985.

The greater efficiency of aerosolization by the particles disclosed herein
5 relative to particles that do not include a surfactant or a charged complex of a
therapeutic agent permits more of a therapeutic agent to be delivered. The use of
biodegradable polymers permits controlled release in the lungs and long-time local
action or systemic bioavailability. Denaturation of macromolecular drugs can be
minimized during aerosolization since macromolecules can be contained and
10 protected within a polymeric shell. Coencapsulation of peptides with peptidase-
inhibitors can minimize peptide enzymatic degradation. Pulmonary delivery
advantageously can eliminate the need for injection. For example, the requirement
for daily insulin injections can be avoided.

The invention is also related to a method for drug delivery to the pulmonary
15 system. The method comprises administering to the respiratory tract of a patient in
need of treatment, prophylaxis or diagnosis an effective amount of particles
comprising a therapeutic, prophylactic or diagnostic agent and a molecule selected
from the group consisting of a surfactant and a molecule having a charge opposite to
the charge of the therapeutic agent and forming a complex thereto. According to one
20 embodiment of the invention the particles have a tap density of less than about 0.4
g/cm³ and a mean diameter of between 5 μ m and 30 μ m. According to another
embodiment of the invention, the non-polymeric particles have a mass density of
less than about 0.4 g/cm³ and a mean diameter of between 5 μ m and 30 μ m. In one
embodiment of the invention, the particles have an aerodynamic diameter between
25 about one and five microns. In another embodiment of the invention, the particles
have an aerodynamic diameter between about one and three microns. In still another
embodiment of the invention, the particles have an aerodynamic diameter between
about three and five microns. In yet another embodiment of the invention, the
particles can be non-polymeric particles.

30 For therapeutic, diagnosis or prophylactic use, particles can be delivered
from an inhaler device, such as a metered-dose-inhaler (MDI), dry-powder inhaler

(DPI) or nebulizer. Such devices are known in the art. For example, a DPI is described in U.S. Patent No. 4,069,819 issued to Valentini, *et al.* on August 5, 1976.

Porous or aerodynamically light particles, having a geometric size (or mean diameter) in the range of 5-30 micrometers, and tap density less than 0.4 g/cm³, such that they possess an aerodynamic diameter of 1-3 micrometers, have been shown to display ideal properties for delivery to the deep lung. Larger aerodynamic diameters are preferred, however, for delivery to the central and upper airways.

Particles having an aerodynamic diameter suited for deposition to the central and upper airways may be delivered as follows. Before or during administration to the respiratory tract, particles having an aerodynamic diameter of about 1-3 microns are aggregated to form aggregated particles which have a larger aerodynamic diameter. According to a preferred embodiment, the aggregated particles have an aerodynamic diameter between approximately three and five microns which is ideal for central airway deposition.

Owing to aggregation, the aerodynamic size range of the aerosol exiting the inhaler and thus the aerodynamic size range of the particles administered to the pulmonary system, can be made to differ from the aerodynamic size range of the airborne particles. Accordingly, aggregated particles can be delivered to the pulmonary system of a patient by an inhaler to achieve aerodynamic diameters in the range of 3-5 micrometers. This method can be used to deliver drugs whose targeted site of action is in the central or upper airways.

The aggregation discussed above is promoted by the chemistry of the powder formulation and by the conditions at which particles exit the inhaler. Non-polymeric particles comprising a therapeutic, prophylactic or diagnostic agent, a surfactant and at least one excipient are preferred. According to one embodiment, the surfactant is DPPC. According to another embodiment, preferred excipients include albumin and lactose.

In addition, aggregation depends on the flow rate at which the particles exit the inhaler. While at high flow rates the particles tend to be fully dispersed, lowering the flow rate promotes aggregation.

EXEMPLIFICATIONS

The present invention will be further understood by reference to the following non-limiting examples.

Example 1: Synthesis of Aerodynamically Light Poly[(*p*-carboxyphenoxy)-
5 hexane anhydride] ("PCPH") Particles

Aerodynamically light poly[(*p*-carboxyphenoxy)-hexane anhydride]
("PCPH") particles were synthesized as follows. 100 mg PCPH (MW~25,000) was
dissolved in 3.0 mL methylene chloride. To this clear solution was added 5.0 mL
1% w/v aqueous polyvinyl alcohol (PVA, MW~25,000, 88 mole % hydrolyzed)
10 saturated with methylene chloride, and the mixture was vortexed (Vortex Genie 2,
Fisher Scientific) at maximum speed for one minute. The resulting milky-white
emulsion was poured into a beaker containing 95 mL 1% PVA and homogenized
(Silverson Homogenizers) at 6000 RPM for one minute using a 0.75 inch tip. After
homogenization, the mixture was stirred with a magnetic stirring bar and the
15 methylene chloride quickly extracted from the polymer particles by adding 2 mL
isopropyl alcohol. The mixture was continued to stir for 35 minutes to allow
complete hardening of the microparticles. The hardened particles were collected by
centrifugation and washed several times with double distilled water. The particles
were freeze dried to obtain a free-flowing powder void of clumps. Yield, 85-90%.

20 The mean diameter of a typical batch prepared by this protocol is 6.0 μm ,
however, particles with mean diameters ranging from a few hundred nanometers to
several millimeters may be made with only slight modifications. Scanning electron
micrograph photos of a typical batch of PCPH particles showed the particles to be
highly porous with irregular surface shape. The particles have a tap density less than
25 0.4 g/cm³.

A surfactant such as DPPC may be incorporated into the polymer solution
prior to particle formation, or optionally the particles can be ionically or covalently
coated by surfactant on the particle surface after particle formation, or the surfactant
may be absorbed onto the particle surface.

Example 2: Synthesis of Spray-Dried Particles**Aerodynamically Light Particles Containing Polymer and Drug Soluble in Common Solvent**

Aerodynamically light 50:50 PLGA particles were prepared by spray drying
5 with testosterone encapsulated within the particles according to the following
procedures. 2.0 g poly (D,L-lactic-co-glycolic acid) with a molar ratio of 50:50
(PLGA 50:50, Resomer RG503, B.I. Chemicals, Montvale, NJ) and 0.50 g
testosterone (Sigma Chemical Co., St. Louis, MO) are completely dissolved in 100
mL dichloromethane at room temperature. The mixture is subsequently spray-dried
10 through a 0.5 mm nozzle at a flow rate of 5 mL/min using a Buchi laboratory spray-
drier (model 190, Buchi, Germany). The flow rate of compressed air is 700 nl. The
inlet temperature is set to 30°C and the outlet temperature to 25°C. The aspirator is
set to achieve a vacuum of -20 to -25 bar. The yield is 51% and the mean particle
size is approximately 5 µm. Larger particle size can be achieved by lowering the
15 inlet compressed air flow rate, as well as by changing other variables. The particles
are aerodynamically light, as determined by a tap density less than or equal to 0.4
g/cm³ and an aerodynamic diameter between one and five microns. Porosity and
surface roughness can be increased by varying the inlet and outlet temperatures,
among other factors.

20 Aerodynamically Light Particles Containing Polymer and Drug in Different Solvents

Aerodynamically light PLA particles with a model hydrophilic drug (dextran)
were prepared by spray drying using the following procedure. 2.0 mL of an aqueous
10% w/v FITC-dextran (MW 70,000, Sigma Chemical Co.) solution was emulsified
into 100 mL of a 2% w/v solution of poly (D,L-lactic acid) (PLA, Resomer R206,
25 B.I. Chemicals) in dichloromethane by probe sonication (Sonics & Materials, Model
VC-250 sonicator, Danbury, CT). The emulsion is subsequently spray-dried at a
flow rate of 5 mL/min with an air flow rate of 700 nl/h (inlet temperature = 30°C,
outlet temperature = 21°C, -20 mbar vacuum). The yield is 56%.

Aerodynamically Light Protein Particles

Aerodynamically light lysozyme particles were prepared by spray drying using the following procedure. 4.75 g lysozyme (Sigma) was dissolved in 95 mL double distilled water (5% w/v solution) and spray-dried using a 0.5 mm nozzle and a Buchi laboratory spray-drier. The flow rate of compressed air was 725 nl/h. The flow rate of the lysozyme solution was set such that, at a set inlet temperature of between 97 and 100°C, the outlet temperature is between 55 and 57°C. The aspirator was set to achieve a vacuum of -30 mbar. The enzymatic activity of lysozyme was found to be unaffected by this process and the yield of the aerodynamically light particles was 66%.

Aerodynamically Light High-Molecular Weight Water-Soluble Particles

Aerodynamically light dextran particles were prepared by spray drying using the following procedure. 6.04 g DEAE dextran (Sigma) was dissolved in 242 mL double distilled water (2.5% w/v solution) and spray-dried using a 0.5 mm nozzle and a Buchi laboratory spray-drier. The flow rate of compressed air was 750 nl/h. The flow rate of the DEAE-dextran solution was set such that, at a set inlet temperature of 155°C, the outlet temperature was 80°C. The aspirator was set to achieve a vacuum of -20 mbar. The yield of the aerodynamically light particles was 66%.

Aerodynamically Light Low-Molecular Weight Water-Soluble Particles

Aerodynamically light trehalose particles were prepared by spray drying using the following procedure. 4.9 g trehalose (Sigma) was dissolved in 192 mL double distilled water (2.5% w/v solution) and spray-dried using a 0.5 mm nozzle and a Buchi laboratory spray-drier. The flow rate of compressed air 650 nl/h. The flow rate of the trehalose solution was set such that, at a set inlet temperature of 100°C, the outlet temperature was 60°C. The aspirator was set to achieve a vacuum of -30 mbar. The yield of the aerodynamically light particles was 36%.

Aerodynamically Light Low-Molecular Weight Water-Soluble Particles

Polyethylene glycol (PEG) is a water-soluble macromolecule, however, it cannot be spray dried from an aqueous solution since it melts at room temperatures below that needed to evaporate water. As a result, PEG was spray-dried at low temperatures from a solution in dichloromethane, a low-boiling organic solvent.

- 5 Aerodynamically light PEG particles were prepared by spray drying using the following procedure. 5.0 g PEG (MW between 15,000 and 20,000, Sigma) was dissolved in 100 mL double distilled water (5.0% w/v solution) and spray-dried using a 0.5 mm nozzle and a Buchi laboratory spray-drier. The flow rate of compressed air 750 nl/h. The flow rate of the PEG solution was set such that, at a
10 set inlet temperature of 45°C, the outlet temperature was between 34 and 35°C. The aspirator was set to achieve a vacuum of -22 mbar. The yield of the aerodynamically light particles (tap density less than 0.4 g/cm³) was 67%.

- A surfactant such as DPPC may be incorporated into the polymer solution prior to particle formation, or optionally the particles can be ionically or covalently
15 coated by surfactant on the particle surface after particle formation, or the surfactant may be absorbed onto the particle surface.

Materials and Methods

The following materials and methods were used in Examples 3 and 4.

Materials

- 20 The polymers: poly(D,L-lactic-co-glycolic acid) (PLGA) with a molar ratio of 50:50 and reported molecular weights of 100,000 Daltons (PLGA RG506) and 34,000 Daltons (PLGA RG503), and poly(D,L-lactic acid) with a reported molecular weight of 100,000 Daltons (PLA R206) were obtained from Boehringer Ingelheim (distributed by B.I. Chemicals, Montvale, NJ). Fluorescently labelled FITC-Dextran
25 with an average molecular weight of 19,000, and L, α -phosphatidylcholine dipalmitoyl (DPPC) were purchased from Sigma Chemical Company, St. Louis, MO.

Microsphere Preparation: Double Emulsion

A double-emulsion solvent-evaporation procedure (Cohen, S., *et al.*, *Pharm. Res.*, 8(6): 713-720 (1991); and Tabata, Y., *et al.*, *Pharm. Res.*, 10(4): 487-496 (1993)), was modified to prepare microspheres for aerosolization. Briefly, 300 µl of an aqueous FITC-Dextran solution (50 mg/ml) was emulsified on ice into a 4.0 ml polymer solution in methylene chloride (200 mg polymer) by sonication at output 3 (Model VC-250, Sonics & Materials Inc., Danbury, CT) using a microtip for 5-10 s to form the inner-emulsion. The first emulsion was poured into 100 ml 1.0 % aqueous PVA solution and homogenized (Model LD4 Homogenizer, Silverson Machines Ltd, England) at 6000 RPM using a 5/8" tip for 1 min to form the double emulsion. The microspheres were continuously stirred for 3 hours to allow hardening, collected by centrifugation, washed several times with double-distilled water, and freeze-dried into a freely flowing powder. Microspheres containing DPPC were prepared by dissolving DPPC in the polymer solution at a concentration of 3 mg/ml prior to the initial emulsification.

15 Microsphere Preparation: Spray Drying

The model hydrophilic drug, dextran labeled with fluorescein isothiocyanate (FITC-Dextran), was encapsulated into PLA or PLGA by a novel emulsion/spray method. For example, 2.0 ml of an aqueous 10% w/v FITC-Dextran (MW=70,000, Sigma Chemical Co.) solution was emulsified into 100 ml of a 2 % w/v solution of PLA in dichloromethane by probe sonication. The emulsion was subsequently spray-dried using a Büchi Mini Spray Drier (Model 190, Büchi Instruments, Germany) at a flow rate of 5 ml/min with an inlet air flow rate of 700 nl/h, inlet temperature of 30°C, outlet temperature of 21°C, and vacuum of - 20 mbar. When DPPC was incorporated it was dissolved in the polymer solution at a concentration of 2 mg/ml prior to emulsification and spray drying.

Microsphere Size Distribution Analysis

Microsphere size distributions were determined using a Coulter Multisizer II (Coulter Electronics Limited, Luton, Beds, England). Approximately 10 drops Coulter type IA non-ionic dispersant were added, followed by 2 mL isoton II solution (Coulter), to 5-10 mg microspheres, and the spheres were dispersed by brief
5 vortex mixing. This suspension was added to 50 mL isoton II solution until the coincidence of particles was between 5 and 8 %. Greater than 500,000 particles were counted for each batch of spheres.

Drug Distribution by Confocal Microscopy

For confocal microscopy, a few milligrams of microspheres containing
10 FITC-Dextran as the drug were suspended in glycerin by brief probe sonication (Vibra-cell Model VC-250 Sonicator, 1/8" microtip probe, Sonics & Materials Inc., Danbury, CT) at output 4 (50W). A drop of the suspension was placed onto a glass slide and a glass cover slip was applied and held in place with finger nail polish. The suspension was allowed to settle for one hour before being viewed by confocal
15 microscopy (Bio-Rad MRC-600 Confocal, Axioplan microscope).

Microsphere Morphology by Scanning Electron Microscopy (SEM)

Microsphere morphology was observed by scanning electron microscopy (SEM) using a Stereoscan 250 MK3 microscope from Cambridge Instruments (Cambridge, MA) at 15 kV. Microspheres were freeze-dried, mounted on metal
20 stubs with double-sided tape, and coated with gold prior to observation.

Microsphere Density Analysis

Microsphere bulk density was estimated by tap density measurements, such as obtained using a Dual Platform Microprocessor Controlled Tap Density Tester (Vankel, NC) and confirmed by mercury intrusion analysis at Porous Materials, Inc.
25 (Ithaca, NY).

Determination of Amount FITC-Dextran and DPPC Encapsulated

The amount of model drug, FITC-Dextran, encapsulated into microspheres was determined by dissolving 10.0 mg microspheres in 3.0 ml 0.8 N NaOH overnight at 37°C, filtering with a 0.45 µm filter (Millipore), and measuring the fluorescence relative to a standard curve (494 nm excitation and 525 nm emission) using a fluorimeter. The drug loading was determined by dividing the amount of FITC-Dextran encapsulated by the theoretical amount if it all were encapsulated. The amount of surfactant, DPPC, encapsulated into microspheres was determined by dissolving 10.0 mg of microspheres in chloroform and using the Stewart Assay (New, R.R.C., "Characterization of Liposomes," in *Liposomes: A Practical Approach*, R. New, Editor, IRL Press, New York, 105-161 (1990)).

In Vitro Aerosolization and Inertial Deposition Behavior

The *in vitro* microparticle aerodynamic characteristics were studied using an Andersen Mark I Cascade Impactor (Andersen Samplers, Atlanta, GA) at an air flow rate of 28.3 l/min. The metal impaction plates were coated with a thin film of Tween 80 minimize particle bouncing Turner, J. and S. Hering, *J. Aerosol Sci.*, 18: 215-224 (1987). Gelatin capsules (Eli Lilly) were charged with 20 mg of microparticles and loaded into a Spinhaler® inhalation device (Fisons, Bedford, MA). The aerosolization experiments were done in triplicate. In each experiment, 10 inhalers were discharged for 30 seconds into the impactor. A 60-second interval was observed between every two consecutive aerosolizations. Fractions of microspheres deposited on each of nine stages, corresponding to stages 0 to 7, and the filter (F) of the impactor, were collected in volumetric flasks by carefully washing the plates with NaOH solution (0.8 N) in order to provide degradation of the polymer and complete dissolution of the fluorescent material. After 12 hours of incubation at 37°C, the solutions were filtered with a 0.45 µm filter and the amount of fluorescent material in each stage was measured at 494 nm (excitation) and 525 nm (emission) using a fluorimeter. Respirable fraction of the delivered dose was calculated according to the fluorescence measurements as percentages of the total fluorescence (i.e., that amount collected in stages 0 to Filter) compared with that collected in stages 2 to Filter of the Impactor.

In Vivo Particle Distribution Following Aerosolization in Rats

Male Sprague Dawley rats (between 150 and 200 g) were anesthetized using a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). The anesthetized rat was placed ventral side up on a surgical table provided with a temperature controlled pad to maintain physiological temperature. The animal was cannulated above the carina with an endotracheal tube connected to a Harvard ventilator (Rodent Ventilator Model 683, South Natick, MA). The animal was force ventilated for 20 minutes at 300 ml/min. 50 mg of microspheres made with or without DPPC were introduced into the endotracheal tube. Following the period of forced ventilation, the animal was sacrificed and the lungs and trachea were separately washed using bronchialveolar lavage as follows: a tracheal cannula was inserted, tied into place, and the airways were washed with 10 ml aliquots of phenol red-free Hanks balanced salt solution (Gibco, Grand Island, NY) without Ca^{2+} and Mg^{2+} (HBSS). The lavage procedure was repeated until a total volume of 30 ml was collected. The lavage fluid was centrifuged (400 g) and the pellets collected and resuspended in 2 ml HBSS. 100 μl was removed for particle counting using a hemacytometer. The remaining solution was mixed with 10 ml of 0.4 N NaOH. After incubation at 37°C for 12 hours, the fluorescence of each solution was measured (wavelengths of 494 nm excitation, 525 nm emission) using a fluorimeter.

Example 3: Fabrication of PLGA microspheres by a Double Emulsion Process Which Encapsulate a Model High-Molecular-Weight Drug, FITC-Dextran.

Scanning electron microscopy "SEM" photographs showing surface morphology of microspheres (MS) made by the double emulsion process with and without the lung surfactant, DPPC were obtained. By SEM, the microspheres made with and without DPPC by the double emulsion process had very similar surface characteristics and size distribution, as confirmed by size distribution measurements, shown below in Table 1.

The efficient entrapment of DPPC within microspheres (83% of theoretical \pm 11% standard deviation, n=6) was confirmed by dissolving an aliquot of MS in

chloroform and detecting the DPPC concentration in solution by the Stewart Assay, as shown in Table 1. Particles made by double emulsion with DPPC are easily resuspended in aqueous solution after lyophilization and are lump-free when dry as determined by light microscopy. Particles made by the double emulsion process
5 without DPPC resuspend easily, however, they appear somewhat agglomerated when dry by light microscopy.

Table 1. Characteristics of Microparticles used for *In Vitro* and *In Vivo* Aerosolization^a

Sample	Mass-Mean (True) Diameter, (μm)	DPPC Load ($\mu\text{g}/\text{mg}$ spheres)	DPPC Loading Efficiency, (%)	FITC-Dextran (Model Drug) Loading Efficiency, (%)
MS without DPPC	8.5 ± 0.76	0	N/A	95.8
MS with DPPC	8.2 ± 0.18	45 ± 6	83 ± 11	82.4

^a Values are given \pm standard deviation.

Confocal microscopy was used to evaluate the distribution of the model drug, FITC-Dextran (M_w 19,000), throughout microspheres made without DPPC and with DPPC. In each case, the drug is evenly dispersed throughout the polymer matrix, which can lead to prolonged delivery of macromolecules after placement in an aqueous environment.

The density of the microspheres as determined by mercury intrusion analysis is shown in Table 2 (and confirmed by tap density measurements).

Table 2. Comparison of Porous Microparticles with Bulk (PLGA 50:50) Polymer

Sample	Density, ρ_{MS} (g/cc)	Respirable Size Range, d_{resp} (μm)
Bulk PLGA	1.35	0.69 - 4.05
MS without DPPC	0.37 ± 0.03	1.3 - 7.7
MS with DPPC	0.30 ± 0.06	1.46 - 8.58

Using the concept of aerodynamic diameter (Gonda, I., in *Topics in Pharmaceutical Sciences 1991*, D. Crommelin and K. Midha, Editors, Stuttgart: Medpharm Scientific Publishers, pp. 95-117 (1992)), it is possible to determine the size range of the microspheres which are theoretically respirable given their mass density, ρ_{MS} . Specifically, it can be shown below in Equation 2 that:

$$\frac{0.8}{\sqrt{\rho_{MS}}} \leq d_{resp} \leq \frac{4.7}{\sqrt{\rho_{MS}}} \quad (2)$$

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where d_{resp} corresponds to the diameter of particles (in μm) theoretically able to enter and remain in the airways without inertial or gravitational deposition (particles smaller than this range are exhaled), and where ρ_{MS} is in units of g/cc. The theoretical respirable size range of the microspheres also is shown in Table 2. The optimal size range (*i.e.*, d_{resp}) for a non-porous PLGA 50:50 microsphere is 0.69-4.05 μm (Table 2). The optimal respirable size range for microspheres without DPPC is 1.3-7.7 μm and, for microspheres with DPPC, 1.46 - 8.58 μm (Table 2). The upper limit on size of respirable particles is increased from 4.05 to greater than 8.5 μm when DPPC is used in the PLGA microsphere preparation. Therefore, the use of low density DPPC microspheres allows the use of larger particles for aerosolization, which may have advantages for drug delivery, such as less particle-particle interaction due to decreased surface area to volume ratio, and lower susceptibility to phagocytosis by alveolar macrophages. In addition, a primary effect of DPPC is to render the particles less adhesive and therefore allow improved aerosolization, as demonstrated below.

Figures 1 and 2 show the results of an *in vitro* aerosolization of the PLGA microspheres made by a double emulsion process with and without DPPC. The microspheres were aerosolized as a dry powder released from a Spinhaler® dry powder inhaler (DPI). Figure 1 illustrates the mass-fraction of the initial dose that is released from the dry powder inhaler device (DPI Efficiency) using an Andersen Mark I Cascade Impactor. DPI efficiencies approaching 80% were obtained with microspheres made with and without DPPC. Although the DPI efficiencies for the two batches were nearly the same, a great difference can be seen between microspheres made with and without DPPC when their deposition within the cascade impactor is observed (Figure 2).

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Figure 2 shows the mass fraction of aerosolized particles that is deposited in stages 2 through Filter (2-Filter) of the Andersen cascade impactor, considered the stages corresponding to the respirable fraction of the microspheres. Stages 0 and 1 correspond roughly to the mouth and throat, and to the upper airways of the lung, respectively. Stages 2-F correspond to successively deeper fractions of the lung. It can be seen that a much greater percentage of microspheres make it to the latter stages of the impactor (considered deeper portions of the lungs) when DPPC is used in their preparation. Overall, greater than 35% (37.0 ± 2.1) of aerosolized particles made with DPPC are considered respirable compared with 13.2 ± 2.9 % without DPPC, as shown in Table 3. The large difference in respirable fraction between the DPPC and non-DPPC particles is at least in part attributed to reduced particle-particle interaction due to the use of DPPC.

In order to estimate the theoretical respirable fraction (RF) of the microspheres, and compare it with experimentally measured *in vitro* and *in vivo* RF's, size distribution measurements were analyzed to determine the percentage of particles (by mass) of each type (DPPC and non-DPPC) that were within the theoretical respirable size range (*i.e.*, d_{resp} Table 2). As shown in Table 3, a higher percentage of particles made with DPPC are expected to be respirable compared with non-DPPC particles (63 to 51%, respectively). This theoretical respirable fraction is based on the mass fraction of microspheres with diameters in the respirable size range, d_{resp} as defined by Eq. (2), and therefore takes into account the different sizes and densities of the two batches of microspheres.

Table 3. Comparison of Microparticle Aerosolization Properties *In Vitro*

Sample	Theoretical Respirable Fraction (i.e., Mass % of microspheres in Respirable Size Range) ^a	Measured Respirable Fraction (% <i>In Vitro</i>) ^b
microspheres without DPPC	51 ± 6	13.2 ± 2.9
microspheres with DPPC	63 ± 2	37.0 ± 2.1

^aBased on theoretical respirable size range (d_{resp} Table 2) and size distribution analyses.

^bMeasured using an Andersen Mark I Cascade Impactor.

To determine whether agglomeration forces during particle aerosolization from the Spinhaler device might be playing a role even after the particles enter the impactor system (i.e., primarily non-DPPC particles remain agglomerated in the inspired stream, resulting in deposition in the first two impactor stages: stages 0 and 5 1), *in vivo* aerosolization experiments were performed in which particles were permitted to fall by gravity into the inspiration stream of a Harvard ventilator system joined with the trachea of an anesthetized rat. In this model, approximately 63% of the inhaled DPPC-PLGA particles deposit in the airways and distal lung regions, whereas 57% of the non-DPPC particles are able to penetrate beyond the trachea in 10 the lungs. These respirable fractions are much nearer to the predicted respirable fractions based upon particle diameter and mass density (Table 3).

Particle aggregation thus is less with DPPC-containing PLGA particles than without DPPC, even though the particles are of similar size and surface morphological features. The use of DPPC thus appears to reduce interparticle 15 attractions, such as van der Waals and electrostatic attractions. It is also possible that the presence of DPPC reduces moisture absorption which may cause particle-particle interaction by capillary forces.

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In addition to the biocompatibility features of DPPC and improvement of surface properties of microspheres for aerosolization, it is possible that the release of DPPC from the slow-eroding PLGA microspheres in the alveolar region of the lungs can more effectively insure the maintenance of normal surfactant fluid composition thereby minimizing the possibility of local toxic side effects. The alveolar surfactant fluid layer is, on average, 10 nm thick (Weibel, E.R., *Morphometry of the Human Lung*, New York: Academic Press (1963).

Example 4: Fabrication of PLGA Microspheres by Spray Drying which
Encapsulate a Model High Molecular Weight Drug, FITC-Dextran.

Microspheres were made by spray drying using a variety of polymeric carriers with and without the incorporation of DPPC. The results are summarized in Table 4.

Table 4. Characterization of Spray Dried Microparticulates

Sample	Mass-Mean (True) Diameter, (μm)	DPPC Load (μg/mg spheres) and Efficiency (%)	FITC-Dextran Loading Efficiency, (%)	% of Surface Coated with DPPC by ESCA
R206+DPPC	5.4	^a	54.9	^a
R206 - DPPC	4.4	-	64.8	-
RG503+DPPC	2.0	62.8	65.2	46.5%
RG503 - DPPC	3.0	-	78.2	-
RG506+DPPC	4.3	89.1	62.7	42-62%
RG506 - DPPC	^b	-	100	-

^aNot Determined

^bNo reliable determination because the powder was highly aggregated.

Aerosolization properties of the microspheres also were examined, as shown in Table 5. Microspheres made by spray drying with and without DPPC have similar size distributions (Table 5) and mass densities (0.49 ± 0.04 g/cc). However, the aerosolization performance of spray-dried aerosols made with and without DPPC is markedly different.

Figure 3 shows that the fraction of low-molecular-weight PLGA RG503 microparticles that are aerosolized from the dry powder inhaler (i.e., the % of particles that simulated inhalation, defined as the DPI Efficiency) is 70.4 % when the particles with DPPC compared with only 46.8 % for particles made without DPPC. Furthermore, the deposition of all types of polymer microparticles following aerosolization into an Andersen impactor is greatly improved using DPPC-coated particles (Table 5). Without the use of DPPC, $\leq 2\%$ of the particles aerosolized reach the latter stages of the impactor (those corresponding to the respirable fraction, stages 2-Filter). On the other hand, a maximum of 25.6% of DPPC-coated microspheres reach stages 2-Filter, as shown in Figure 4. Higher respirable fractions may be obtained with particles that contain low molecular weight drugs that are soluble in methylene chloride and therefore do not require the use of water during their preparation.

Table 5. Summary of Aerosolization Data of microspheres Prepared by Spray Drying with or without DPPC

Sample	% Aerosolized Particles that reach stages 1 - Filter	% Aerosolized Particles that reach stages 2 - Filter	% Aerosolized Particles that reach stages 3 - Filter	DPI Efficiency
R206 + DPPC	40.4 \pm 8.4	25.6 \pm 2.3	18.0 \pm 2.7	38.6 \pm 3.7
R206 - DPPC	7.4 \pm 2.1	1.8 \pm 0.5	1.1 \pm 0.3	41.0 \pm 4.8
RG503+DPPC	36.0 \pm 9.2	14.7 \pm 1.53	10.4 \pm 0.46	70.4 \pm 2.4
RG503 - DPPC	3.3 \pm 0.6	2.1 \pm 0.3	2.0 \pm 0.3	46.8 \pm 8.0
RG506+DPPC	13.7 \pm 9.1	7.1 \pm 4.1	4.1 \pm 2.5	76.6 \pm 8.4
RG506 - DPPC	1.8 \pm 0.6	1.6 \pm 0.6	1.4 \pm 0.7	74.0 \pm 7.2

R206 = PLA, molecular weight approximately 100,000.

RG503 = PLGA 50:50, molecular weight approximately 34,000.

RG506 = PLGA 50:50, molecular weight approximately 100,000.

Example 5: Fabrication of Estradiol-Containing Lactose:DPPC Particles.

Materials and Methods: A Niro Atomizer Portable Spray Dryer (Model #68) was used for all of the following Examples. Compressed air with variable pressure ran a rotary atomizer located above the dryer. Liquid feed with varying rate was
5 pumped continuously by an electronic metering pump (LMI, model #A151-192s) to

the atomizer. Both inlet and outlet temperatures can be measured and controlled manually. A container was tightly attached to the cyclone to collect the spray dried powder product.

Estradiol-containing particles were prepared to illustrate the preparation of large porous particles that contain a relatively large drug fraction by weight. Estradiol particles of standard mass density (greater than 0.4 g/cc) can be made in various ways. In this example, the particles included 30% β -estradiol, 62% lactose and 8% DPPC by weight. The lactose was dissolved in deionized water and the estradiol and DPPC were dissolved in 95% v/v ethanol. The two solutions were combined to form an 85% v/v ethanol solution. The total concentration of powdered starting materials in the solution was 3.25% w/v. The solution was spray dried under the following condition: The inlet temperature was 160°C; the outlet temperature was 95°C; the atomization pressure was 2 kp/cm² (28.45 psi); and the feed rate was 34 ml/min. The resulting spray dried powder had a tap (mass) density of 0.46 g/ml. The mean diameter based on volume, as measured using a Microtrac particle sizer, was 3.5 μ m, thus giving an aerodynamic diameter of 2.4 μ m.

In another example, estradiol particles of standard mass density (about 1 g/cc) were prepared by spray drying a solution containing 70% estradiol and 30% DPPC with a total powder concentration of 1.9% w/v in 85% v/v ethanol. The spray dryer was operated under the following conditions; the inlet temperature was 150°C, the outlet temperature was 85°C, the atomization pressure was 1 kp/cm² (14.22 psi), and the feed rate was 30 ml/min. The particles produced had a tap density of 0.62 g/ml and a mean diameter of 6 μ m, thus giving an approximate aerodynamic diameter of 4.7 μ m.

In order to produce light, porous particles, many combinations of operating conditions and powder compositions were tested. Another example of the preparation of low density particles was as follows; A solution of 90% β -estradiol and 10% DPPC by weight in 95% ethanol was prepared. The solution was then combined with deionized water to make a solution of 85% ethanol. The total powder concentration was 1.1% w/v. The operating conditions were as follows; the inlet temperature was 110°C, the outlet temperature was 85°C, the atomization

pressure was 1 kp/cm² (14.22 psi), and the feed rate was 30 ml/min. The yield was 53.0%. The resulting powder was very flowable, and was made up of particles possessing irregular shapes and rough surfaces, as viewed by a SEM (scanning electron microscope). The mean diameter, determined by the Microtrac, based on volume was 6 µm. The tap density was 0.28, thus giving an approximate aerodynamic diameter of 2.6 microns, which falls within the desired range of between one and five microns.

Example 6: Preparation of Lactose:DPPC Carrier Particles.

"Carrier" particles can be created to mimic drug-carrying particles with similar excipient concentrations. Case studies of four carrier particles are discussed below, followed by two examples of adding small concentrations of drug to the carrier particle. In this example, a small weight percentage of drug in the particle is considered to be less than 20% of the total powder weight.

Carrier particles with standard mass density can be prepared via several methods. An example is the following formulation. Solution of lactose in deionized water and DPPC in ethanol were combined to provide a solution containing relative ratios of 67% lactose and 33% DPPC by weight in 85% ethanol, with the total powder concentration in the solution of about 0.1% w/v. The solution was spray dried under the following conditions; the inlet temperature was 200°C; the outlet temperature was 119°C; the atomization pressure was 3 kp/cm² (42.72 psi); and the feed rate was 40 ml/min. The yield of this run was 29.3%. The resulting spray dried powder had a tap (mass) density of 0.41 g/ml and a mean diameter by volume average estimated from an SEM of 2.5 µm, thus giving an approximated aerodynamic diameter of 1.6 microns, which is within the desired range of between one and five microns.

Powder composition, powder concentration, solvent composition and spray drier operating conditions are some of the factors which can be varied in order to produce light, porous carrier particles. Large, porous particles can be made that have a donut-like morphology. Such particles can be prepared, for example, by preparing a solution that includes 33% human albumin, 33% lactose, and 33% DPPC by

weight. The human albumin and lactose was dissolved in deionized water and the DPPC was dissolved in 95% ethanol. The two solutions were combined to yield an 85% ethanol solution. The total powder concentration was about 0.1% w/v. The solution was spray dried under the following conditions; the inlet temperature was 110°C; the outlet temperature was 60°C; the atomization pressure was 3 kp/cm² (42.72 psi); and the feed rate was 40 ml/min. The yield from this run was 38.5%. The tap (mass) density of the resulting particles was 0.16 g/ml, and the size of this particle on the coulter counter is 7.6 µm, thus giving an approximate aerodynamic diameter of 3.0 µm. (Note: The volume average sizes approximated from the SEM and those determined by the Coulter Counter can be considered equivalent.)

Example 7: Preparation of Albumin:Lactose:DPPC Particles.

Another type of large, porous particles looks similar to a dried grape. Particles with this type of morphology can be prepared, for example, by spray drying a solution that contains 20% human albumin, 20% lactose, and 60% DPPC by weight. The human albumin and lactose were dissolved in deionized water and the DPPC was dissolved in 95% ethanol. The two solutions were combined to form an 85% ethanol solution. The total powder concentration was about 0.1% w/v. The solution was spray dried under the following conditions; the inlet temperature was 110°C; the outlet temperature was 60°C; the atomization pressure was 3 kp/cm² (42.72 psi); and the feed rate was 40 ml/min. The yield was 45.0%. The tap (mass) density of this particle is 0.05 g/ml, and the approximate volume-average size of this particle from the SEM was 7 µm, thus giving an approximate aerodynamic diameter of 1.6 µm. Aerosolization studies of this particle yielded the following results; aerosolized fraction was 58.5%; respirable fraction was 26.6%, and respirable fraction of inhaled aerosol was 43.8%.

Example 8: Preparation of Albumin:Lactose:DPPC Particles.

Various methods can be used to increase the size of the particles. The particles prepared in this example had roughly the same morphology as those in Example 7, but had a larger particle size. The particles were prepared as follows: A

solution of 20% human albumin, 20% lactose, and 60% DPPC by weight was spray dried. The human albumin and lactose were dissolved in deionized water and the DPPC was dissolved in 95% ethanol. The two solutions were combined to form an 85% ethanol solution. The total powder concentration was about 0.2% w/v. The solution was spray dried under the following conditions; the inlet temperature was 110°C; the outlet temperature was 51°C; the atomization pressure was 2 kp/cm² (28.48 psi); and the feed rate was 66 ml/min. The yield from this run was 48.6%. The tap (mass) density of the resulting particles was 0.04 g/ml, and the approximate volume-average size of the particles from the SEM was 10 µm, thus giving an approximate aerodynamic diameter of 2.0 microns.

Example 9: Spray Drying of Insulin:Albumin:Lactose:DPPC Particles.

This example demonstrates that adding less than 20% drug by weight has little change on the particle morphology, size, tap density, and aerosolization characterizations. For example, human insulin was added at a concentration of about 2% by weight of the particles in Example 7. The particles were prepared by spray drying a solution of 2% human insulin, 19% human albumin, 19% lactose, and 60% DPPC by weight. The human insulin, human albumin and lactose were dissolved in deionized water and the DPPC was dissolved in 95% ethanol. The solubility of human insulin in the deionized water was increased by adding a few drops of NaOH (5g NaOH/100 ml deionized water) until the insulin went into solution. The two solutions were combined to form an 85% ethanol solution. The total powder concentration was about 0.1% w/v. The solution was spray dried under the following conditions; the inlet temperature was 110°C; the outlet temperature of 61°C; the atomization pressure was 3 kp/cm² (42.72 psi); and the feed rate was 40 ml/min. The yield from this run was 51.1%. The tap (mass) density of the resulting particles was 0.05 g/ml and the approximate volume-average size of this particle from the SEM was 6.5 µm, thus giving an approximate aerodynamic diameter of 1.5 µm. The morphology of the particles was very similar to the particles in Example 7. Aerosolization studies of these particles yielded the following results: the

aerosolized fraction was 45.0%; the respirable fraction was 15.0%; the respirable fraction of the inhaled aerosol was 58.3%.

Example 10: Preparation of Albuterol Particles.

Albuterol particles with a relatively small amount of drug by weight were also prepared. In this example, particles were prepared according to the procedure in Example 6, except that 4% albuterol by weight of the particle was added. The particles were formed by spray drying a solution containing 4% albuterol, 33% human albumin, 33% lactose, and 33% DPPC by weight. The albuterol, human albumin and lactose were dissolved in deionized water and the DPPC was dissolved in 95% ethanol. The solutions were combined to form an 85% ethanol solution. The total powder concentration was about 0.1% w/v. The solution was spray dried under the following conditions; the inlet temperature was 110°C; the outlet temperature was 60°C; the atomization pressure was 3 kp/cm² (42.72 psi); and the feed rate was 40 ml/min. The yield from this run was 46.8%. The tap (mass) density of the resulting particles was 0.15 g/ml and the size of the particles as measured on a Coulter counter was 7.2 µm, thus giving an approximate aerodynamic diameter of 2.8 µm.

Example 11: Preparation of Sustained Release Insulin Particles.

Sustained release of insulin out of the particles was achieved by rendering the insulin insoluble. Insulin was dissolved in ultrapure water (0.02% w/v). Protamine was then added (in the proportion insulin/protamine 5/1 w/w) to form an insulin/protamine complex. The formation of the insulin/protamine complex causes the insulin to precipitate. The complex was dissolved by raising the pH to about 5 with HCl so that the solution could be spray dried. Lactose was then added to the solution. The aqueous solution was then mixed with a 95% v/v ethanol solution containing DPPC. The final concentration of each excipient in the 85% v/v solution was insulin/protamine/lactose/ DPPC 2/0.4/37.6/60% w/v. The solution was spray dried under the following conditions; the inlet temperature was 110°C; the outlet

temperature was 60°C; the atomization pressure was 3 kp/cm² (42.72 psi); and the feed rate was 40 ml/min. The ability of the particles to provide sustained release *in vitro* was evaluated. Particles suspended in phosphate buffer saline at pH 7.4 released less than 10% of the incorporated insulin after 5 hours.

Example 12: Preparation of Insulin:Protamine:Zinc Complexes.

Particles containing a complex of insulin/protamine/zinc were prepared according to the process in Example 11. The concentration of each excipient in the ethanol/water (85:15 % v/v) solution was insulin/protamine/zinc chloride/lactose/DPPC 2:0.6:0.25:32.4:60 (% w/v). The solution was spray dried under the same conditions in Example 11. The formulation was also shown to provide sustained release of insulin *in vitro*.

The particles (8 mg) were inhaled into the lungs of rats using the procedures described in Edwards, et al. (*Science*, 276, 1868 (1997)). For comparison purposes, the particles were also injected subcutaneously and non-sustained insulin particles of identical insulin content (without protamine or zinc) were injected subcutaneously and inhaled. Figure 5 shows the plasma concentration per unit time for insulin administered via the various modes of administration. The inhaled protamine/zinc particles resulted in sustained high serum insulin concentrations for at least 24 hours, in contrast to particles without protamine or zinc, which released insulin in less than approximately 5 hours.

Therapeutics other than insulin can be complexed in the same manner and included in the particles. Proteins having an isoelectric point (pI) lower than the physiological pH of 7.4 as insulin (pI=5.3) can be precipitated in the same manner using protamine (e.g., growth hormone, pI=4.9). Proteins having a pI higher than pH of 7.4 (e.g., LHRH, calcitonin) can be precipitated using a negatively charged compound (e.g., dextran-sulfate) or by adding an appropriate salt. This approach can be extended to drugs (e.g., heparin) other than therapeutic proteins as well.

Example 13: Preparation of Sustained Release Albuterol Particles.

Albuterol particles were prepared to evaluate the sustained release of a hydrophilic molecule from particles. The albuterol-containing particles were prepared as described in Example 7, reducing the percentages of lactose and albumin (while keeping the ratio equal) and adding cholesterol (of varying percentages; 6, 8, 10, 25%) and albuterol (4%). The addition of cholesterol led to an increasingly slower release of albuterol, as shown in Figure 6. Albuterol concentration was measured using a UV spectrophotometer. The data shown in Figure 6 demonstrates that cholesterol can be incorporated into particles to provide sustained albuterol release. Similar results can be achieved by increasing the DPPC concentration beyond 60%.

Example 14: Release Properties of Albumin:DPPC:Lactose:Albuterol Particles.

Particles (mean diameter 10 μ m, tap density 0.06 gram³) were prepared particles as described in Example 7 with 60% DPPC, 18% albumin, 18% lactose, and 4% albuterol to demonstrate that sustained release of a hydrophilic molecule such as albuterol can also be achieved without cholesterol. The *in vitro* release of albuterol is shown in Figure 7 both for this formulation and a non-sustained release formulation that included only lactose (96%) and albuterol (4%). Even without cholesterol, the release of the albuterol was sustained for nearly 24 hours.

Particles (5 mg, i.e. 200 μ g albuterol dose) were administered to guinea pigs using the procedures in Example 12 to demonstrate that the sustained release albuterol particles could produce sustained bronchodilation. The animals were administered carbachol prior to measuring airway resistance. Airway resistance was monitored using a Buxco system. Airway resistance dropped sharply following inhalation of the large porous particles (Figures 7 and 8) and remained at statistically low levels for approximately 1 day (n = y).

"Placebo" particles (60% DPPC, 20% albumin, 20% lactose) prepared as described in Example 11 were also administered. Airway resistance following carbachol challenge was measured at eight hours following inhalation and 15 hours following inhalation. The airway resistance was 1.0 ± 0.3 and 1.0 ± 0.2 cm

H₂O/ml/sec., proving that the bronchodilation observed in Figure 8 was due to slow albuterol release.

Slow albuterol release has also been achieved *in vitro* using particles prepared by the methods of Example 7 with 10% DPPC, 86% albumin, and 4% albuterol. However particles prepared with 10% DPPC, 43% albumin, 43% lactose, and 4% albuterol did not display significantly slower albuterol release *in vitro*, indicating that for relatively low DPPC content, high albumin content is favorable for sustained albuterol release.

These examples demonstrate that by choosing the composition of the spray dried materials and by varying the spray drying parameters, the aerodynamic properties of the inhaled particles can be effectively controlled. More specifically, the composition of the spray dried material especially affects the density and shape of the particles while the spray drying parameters have a stronger affect on their size. For instance, increasing the proportion of lactose in the particles make the particles heavier, while increasing the albumin or dipalmitoyl phosphatidylcholine (DPPC) content makes them lighter. Increasing DPPC content also increases the particle size. Nevertheless, when a relatively small proportion of drug is incorporated in the particles, the characteristics of the particles remain relatively unaffected. Decreasing the inlet temperature largely increases the size of the particles without greatly affecting their tap density. Increasing the feed rate and decreasing the pressure of the compressed air both tend to increase the size of the particles without greatly affecting their density. However, these effects are smaller than those of the temperature.

Example 15: Aggregated Particles Comprising Albuterol

Large porous or aerodynamically light albuterol particles were produced by spray drying a cosolvent mixture of ethanol and water containing a dissolved solute concentration of 0.1%. The dry particles or powders were spray dried using a Niro portable spray drier (NIRO, Columbia, MD), and contained 4% by weight albuterol sulfate, 18% by weight human serum albumin, 18% by weight lactose, and 60%by weight dipalmitoyl phosphatidylcholine (dppc). The tap density was measured using

a Dual Platform Microprocessor Controlled Tap Density Tester (Vankel, NC), which is a USP tap density method. The tap density of the powder was 0.05 g/cc. The geometric size distribution was measured using a Coulter Multisizer IIe. The average geometric diameter was 10 micrometers. The theoretical average aerodynamic diameter corresponding to the measured tap density and the measured geometric size was 2.2 micrometers, which was less than or equal to 3 micrometers and ideal for inhalation to the peripheral airways or alveoli. The average aerodynamic diameter of the dry powder was measured experimentally by a time of flight device Aerosizer and Aerodisperser (API, Amherst) as a function of increasing shear rate, to identify the minimum average aerodynamic diameter of the powder (i.e., in its fully deaggregated state). The average aerodynamic diameter did not change in the range of 1-5 psi, indicating that the powder was in its fully deaggregated state. As illustrated in Figure 9, this was found to be 2.9 micrometers. Parameters related to the data shown in Figure 9 are tabulated in Tables 6 and 7.

Table 6

PARAMETERS	DISPERSER CONTROL	%UNDER	SIZE	%UNDER	SIZE
Material:Aerodynamic	Disperser Type: AeroDisperser	5%	1.350	55%	3.005
Density : 1.00	Shear Force: 1.0 0.1 psi	10%	1.662	60%	3.129
Run Length (sec): 57.0	Feed rate: 5000, 1000	15%	1.884	65%	3.253
PMTVoltage(volts):1100.0		20%	2.070	70%	3.388
Laser Current (mA): 39.2	Deagglomeration: Normal	25%	2.232	75%	3.527
Clock Freq (MHz): 40.0	Pin Vibration: On	30%	2.377	80%	3.686
Sum of Channels: 187670		35%	2.511	85%	3.894
Lower Size Limit: 0.50		40%	2.637	90%	4.154
Upper Size Limit: 998.00		45%	2.763	95%	4.493
Nozzle Type: 700 micron		50%	2.888		
Baseline Offset: 0.10					
NoiseFilter: 6.00	SCANS 25 and 26 Combined Between 2.6 and 2.6 microns				
Mean Size : 2.718	D(4,3) : 2.895	Mode (Linear Scale): 3.00			
Standard Deviation: 1.457	D(3,2) : 2.507	Spec Surf Area: 2.39 sq. meter/g			

Table 7

Upper % Size In Under	Lower % Size Under	Upper % Size In Size Under	Lower % Size Under	Upper % Size In Size Under	Lower % Size Under	Upper % Size In Size Under	Lower % Size Under
	501 0.0000 431 100.00	50.1 0.0000 43.1 100.00	5.01 5.9141 4.31 92.551				
	431 0.0000 371 100.00	43.1 0.0000 37.1 100.00	4.31 11.947 3.71 80.604				
	371 0.0000 316 100.00	37.1 0.0000 31.6 100.00	3.71 19.472 3.16 61.132				
	316 0.0000 271 100.00	31.6 0.0000 27.1 100.00	3.16 18.301 2.71 42.831				
	271 0.0000 231 100.00	27.1 0.0000 23.1 100.00	2.71 15.353 2.31 27.478				
	231 0.0000 200 100.00	23.1 0.0000 20.0 100.00	2.31 9.3203 2.00 18.157				
	200 0.0000 170 100.00	20.0 0.0000 17.0 100.00	2.00 7.3191 1.70 10.838				
	170 0.0000 145 100.00	17.0 0.0000 14.5 100.00	1.70 4.4607 1.45 6.3776				
	145 0.0000 125 100.00	14.5 0.0000 12.5 100.00	1.45 2.4335 1.25 3.9441				
	125 0.0000 110 100.00	12.5 0.0000 11.0 100.00	1.25 1.2725 1.10 2.6716				
	110 0.0000 90.2 100.00	11.0 0.0000 9.02 100.00	1.10 1.1949 0.90 1.4768				
	90.2 0.0000 80.2 100.00	9.02 0.0000 8.02 100.00	0.90 0.4823 0.80 0.9945				
	80.2 0.0000 70.2 100.00	8.02 0.0000 7.02 100.00	0.80 0.4164 0.70 0.5781				
702 0.0000 601 100.00	70.2 0.0000 60.1 100.00	7.02 0.0000 6.01 100.00	0.70 0.3448 0.60 0.2332				
601 0.0000 501 100.00	60.1 0.0000 50.1 100.00	6.01 0.0000 5.01 100.00	0.60 0.2490 0.50 0.0000				

The powder was then placed in a #2 hard gelatin capsule and loaded inside a DPI such as that described in U.S. Patent 4,069,819, issued on August 5, 1978 to Valentini et al. The powder was inspired into the Aerosizer at a flow rate of 30 L/min, characteristic of a normal breath. Also shown in Figure 9 is the aerodynamic size distribution following exit of the powder from the DPI. The average aerodynamic diameter of the powder that exited the inhaler was 3.8 micrometers, which is larger than 2.7 micrometers owing to powder aggregation in the inhaler. The larger average aerodynamic diameter following delivery from the DPI may be used to advantage for drugs whose targeted site of action is in the central or upper airways. Albuterol is such a drug. By adjusting particle chemistry and inhaler parameters, average aerodynamic diameter values in the range of 3-5 micrometers,

or 3-10 micrometers, could be obtained, even though the average aerodynamic diameter of the original powder was less than 3 micrometers.

Example 16: Aggregated Particles Comprising Estradiol

5 Large porous or aerodynamically light estradiol particles were produced by spray drying a cosolvent mixture of ethanol and water containing a dissolved solute concentration of 0.3%. The particles or powders were spray dried using a Niro portable spray drier (NIRO, Columbia, MD), and contained 90% by weight estradiol and 10% by weight dppc. The tap density was measured using a Dual Platform
10 Microprocessor Controlled Tap Density Tester (Vankel, NC), which is a USP tap density method. The tap density of the powder was 0.08 g/cc. The geometric size distribution was measured using a Coulter Multisizer. The average geometric diameter was 10 micrometers. The theoretical average aerodynamic diameter corresponding to these values for tap density and geometric size was 2.8
15 micrometers, which was less than or equal to 3 micrometers and ideal for inhalation to the peripheral airways or alveoli. The average aerodynamic diameter of the dry powder was measured by Aerosizer used in Example 15 as a function of increasing shear rate, to identify the minimum average aerodynamic diameter of the powder (i.e. in its fully deaggregated state). The results of this study are summarized in Fig.
20 10. Parameters related to the data shown in Figure 10 are tabulated in Tables 8 and 9. At all shear rates the average aerodynamic diameter was near 3 micrometers. The powder was then placed in a #2 hard gelatin capsule and loaded inside the DPI, as described in Example 15. The powder was inspired into the Aerosizer at flow rates of 10, 15 and 30 L/min, characteristic of slow to normal breathing. Figure 11
25 summarizes the aerodynamic size distribution following exit of the powder from the DPI. Parameters related to the data shown in Figure 11 are tabulated in Tables 10 and 11. The average aerodynamic diameter was near 5 micrometers at the slowest flow rate, and near to the deaggregated powder average aerodynamic diameter of 3 micrometers at or above a flow rate of 15 L/min. This demonstrated that deep lung
30 deposition can be achieved with the large porous estradiol particles when they are inhaled into the lungs from the inhaler at a flow rate of 30 L/min or more;

alternatively, if more central deposition is desired, inhalation of the particles from the inhaler at a flow rate of around 10 L/min results in sufficient aggregation to achieve this pattern of deposition.

Large porous powders with average aerodynamic diameter values in the 1-3 5 micrometer range may therefore be inhaled into the lungs either with an average aerodynamic diameter in this range or with an average aerodynamic diameter larger than this range, depending on the needs of the therapy, via use of the powders in an inhaler device, and appropriate design of the powders and device.

Table 8

PARAMETERS	DISPERSER CONTROL	%UNDER	SIZE	%UNDER	SIZE
Material:Aerodynamic	Disperser Type: AeroDisperser	5%	1.468	55%	3.524
Density : 1.00	Shear Force: 1.0 0.1 psi	10%	1.849	60%	3.725
Run Length (sec): 111.5	Feed rate: 5000, 1000	15%	2.088	65%	3.925
PMTVoltage(volts):1100.0		20%	2.284	70%	4.133
Laser Current (mA): 39.7	Deagglomeration: Normal	25%	2.461	75%	4.361
Clock Freq (MHz): 40.0	Pin Vibration: On	30%	2.634	80%	4.616
Sum of Channels: 525388		35%	2.806	85%	4.943
Lower Size Limit: 0.50		40%	2.978	90%	5.415
Upper Size Limit: 998.00		45%	3.153	95%	6.108
Nozzle Type: 700 micron		50%	3.336		
Baseline Offset: 0.10					
NoiseFilter: 6.00	SCANS 293 and 294 Combined				
	Between 2.5 and 2.5 microns				
Mean Size : 3.204	D(4,3) : 3.494	Mode (Linear Scale):		2.91	
Standard Deviation: 1.544	D(3,2) : 2.890	Spec Surf Area:		2.08 sq. meter/g	

Table 9

Upper % Lower % Upper % Lower % Upper % Lower % Upper % Lower %
 Size In Size Under Size In Size Under Size In Size Under Size In Size
 Under

	501	0.0000	431	100.00	50.1	0.0000	43.1	100.00	5.01	11.914	4.31	73.943	
	431	0.0000	371	100.00	43.1	0.0000	37.1	100.00	4.31	14.342	3.71	59.601	
	371	0.0000	316	100.00	37.1	0.0000	31.6	100.00	3.71	14.464	3.16	45.137	
	316	0.0000	271	100.00	31.6	0.0000	27.1	100.00	3.16	13.031	2.71	32.106	
	271	0.0000	231	100.00	27.1	0.0000	23.1	100.00	2.71	11.514	2.31	20.592	
	231	0.0000	200	100.00	23.1	0.0000	20.0	100.00	2.31	7.4742	2.00	13.118	
	200	0.0000	170	100.00	20.0	0.0000	17.0	100.00	2.00	5.4116	1.70	7.7061	
	170	0.0000	145	100.00	17.0	0.0000	14.5	100.00	1.70	2.8440	1.45	4.8621	
	145	0.0000	125	100.00	14.5	0.0000	12.5	100.00	1.45	1.6988	1.25	3.1633	
	125	0.0000	110	100.00	12.5	0.0000	11.0	100.00	1.25	1.0633	1.10	2.1000	
	110	0.0000	90.2	100.00	11.0	0.0000	9.02	100.00	1.10	1.1471	0.90	0.9528	
	90.2	0.0000	80.2	100.00	9.02	0.1435	8.02	99.857	0.90	0.4143	0.80	0.5385	
	80.2	0.0000	70.2	100.00	8.02	1.0966	7.02	98.760	0.80	0.2865	0.70	0.2520	
702	0.0000	601	100.00	70.2	0.0000	60.1	100.00	7.02	4.2793	6.01	94.481	0.70	0.1715
601	0.0000	501	100.00	60.1	0.0000	50.1	100.00	6.01	8.6233	5.01	85.857	0.60	0.0850

Table 10

PARAMETERS DISPERSER CONTROL %UNDER SIZE %UNDER
SIZE

Material:Aerodynamic	Disperser Type: AeroBreather	5%	2.393	55%	5.965
Density : 1.00	Breath (1/min): 10.0	10%	2.868	60%	6.329
Run Length (sec): 64.9	Breath Vol (1): 1.0	15%	3.277	65%	6.714
PMTVoltage(volts):1100.0	Acceleration: 1.0	20%	3.636	70%	7.138
Laser Current (mA): 39.2		25%	3.960	75%	7.638
Clock Freq (MHz): 40.0		30%	4.274	80%	8.214
Sum of Channels: 921984		35%	4.586	85%	8.838
Lower Size Limit: 0.50		40%	4.914	90%	9.610
Upper Size Limit: 998.00		45%	5.255	95%	10.36
Nozzle Type: 700 micron		50%	5.609		
Baseline Offset: 0.10					
NoiseFilter: 6.00	SCANS 301 and 302 Combined Between 11.1 and 11.3 microns				
Mean Size : 5.374	D(4,3) : 5.901	Mode (Linear Scale): 4.47			
Standard Deviation: 1.567	D(3,2) : 4.829	Spec Surf Area: 1.24 sq. meter/g			

Table 11

Upper % Lower % Upper % Lower % Upper % Lower % Upper % Lower %
 Size In Size Under Size In Size Under Size In Size Under Size In Size
 Under

	501	0.0000	431	100.00	50.1	0.0000	43.1	100.00	5.01	10.854	4.31	30.592	
	431	0.0000	371	100.00	43.1	0.0000	37.1	100.00	4.31	9.4999	3.71	21.093	
	371	0.0000	316	100.00	37.1	0.0000	31.6	100.00	3.71	7.6388	3.16	13.454	
	316	0.0000	271	100.00	31.6	0.0000	27.1	100.00	3.16	5.2416	2.71	8.2122	
	271	0.0000	231	100.00	27.1	0.0000	23.1	100.00	2.71	4.0234	2.31	4.1888	
	231	0.0000	200	100.00	23.1	0.0000	20.0	100.00	2.31	2.3081	2.00	1.8807	
	200	0.0000	170	100.00	20.0	0.0000	17.0	100.00	2.00	1.2094	1.70	0.6713	
	170	0.0000	145	100.00	17.0	0.0000	14.5	100.00	1.70	0.3825	1.45	0.2888	
	145	0.0000	125	100.00	14.5	0.0000	12.5	100.00	1.45	0.1516	1.25	0.1372	
	125	0.0000	110	100.00	12.5	1.2321	11.0	98.768	1.25	0.0673	1.10	0.0699	
	110	0.0000	90.2	100.00	11.0	12.590	9.02	86.178	1.10	0.0486	0.90	0.0213	
	90.2	0.0000	80.2	100.00	9.02	7.7222	8.02	78.455	0.90	0.0114	0.80	0.0099	
	80.2	0.0000	70.2	100.00	8.02	9.8438	7.02	68.612	0.80	0.0061	0.70	0.0038	
702	0.0000	601	100.00	70.2	0.0000	60.1	100.00	7.02	12.923	6.01	55.689	0.70	0.0028
601	0.0000	501	100.00	60.1	0.0000	50.1	100.00	6.01	14.242	5.01	41.447	0.60	0.0011

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

CLAIMS

What is claimed is:

- 5 1. A method for drug delivery to the pulmonary system comprising:
administering to the respiratory tract of a patient in need of treatment,
prophylaxis or diagnosis an effective amount of particles comprising a
therapeutic, prophylactic or diagnostic agent and a material selected from the
group consisting of surfactant and a molecule having a charge opposite to the
10 charge of the agent and forming a complex thereto, said particles having a tap
density less than about 0.4 g/cm³, a mean diameter of between 5 µm and 30
µm and an aerodynamic diameter of between approximately one and three
microns,
wherein, before or during administering to the respiratory tract, the
15 particles are aggregated to form aggregated particles, said aggregated
particles having an aerodynamic diameter between approximately three and
five microns.
2. The method of Claim 1 wherein the agent includes albuterol.
- 20 3. The method of Claim 1 wherein the agent includes estradiol.
4. The method of Claim 1 wherein the surfactant includes a phosphoglyceride.
- 25 5. The method of Claim 4 wherein the surfactant includes dipalmitoyl L- alpha-
phosphatidylcholine.
6. The method of Claim 1 wherein the surfactant is endogenous to the lung.
- 30 7. The method of Claim 1 wherein the complexed agent includes insulin:
protamine:zinc complex.

8. Aggregated particles for drug delivery to the pulmonary system having an aerodynamic diameter between approximately three and five microns and including particles comprising a therapeutic, diagnostic or prophylactic agent and a surfactant, wherein, particles have a tap density less than about 0.4 g/cm³, a mean diameter of between 5 μ m and 30 μ m and an aerodynamic diameter of between approximately one and three microns.
5
9. The particles of Claim 8 wherein the agent has a charge and is complexed with a molecule having an opposite charge to the charge of the agent.
10
10. The particles of Claim 9 wherein the complexed agent includes insulin: protamine:zinc.
11. The particles of Claim 8 wherein the agent includes albuterol.
15
12. The particles of Claim 8 wherein the agent includes estradiol.
13. The particles of Claim 8 wherein the surfactant includes a phosphoglyceride.
- 20 14. The particles of Claim 8 wherein the surfactant includes dipalmitoyl L-alpha-phosphatidylcholine.
15. The particles of Claim 8 wherein the surfactant includes a surfactant endogenous to the lung.
25
16. Aggregated particles for drug delivery to the pulmonary system having an aerodynamic diameter between approximately three and five microns and including particles comprising a charged therapeutic, diagnostic or prophylactic agent and a molecule having a charge opposite to the charge of the agent and forming a complex thereto, wherein the particles have a tap
30

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density less than 0.4 g/cm^3 , a mean diameter between $5 \text{ }\mu\text{m}$ and $30 \text{ }\mu\text{m}$ and aerodynamic diameter between one and three microns.

17. The particles of Claim 16 wherein the complexed agent includes insulin:
5 protamine:zinc complex.
18. The particles of Claim 16 further including a surfactant.

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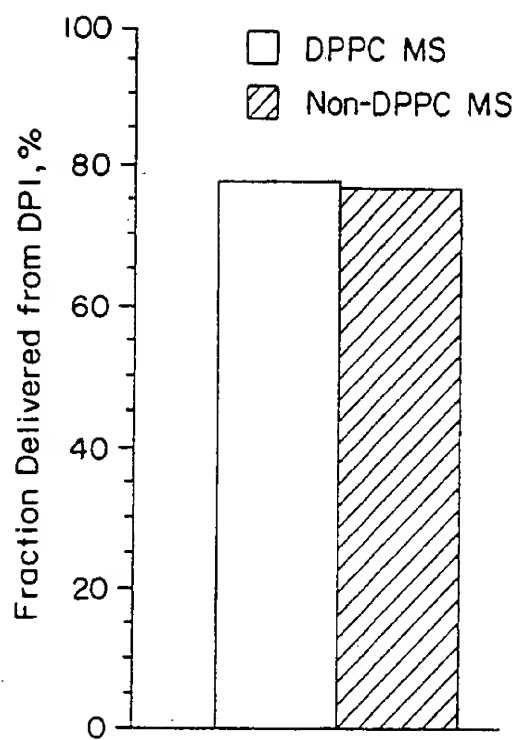


FIG. 1

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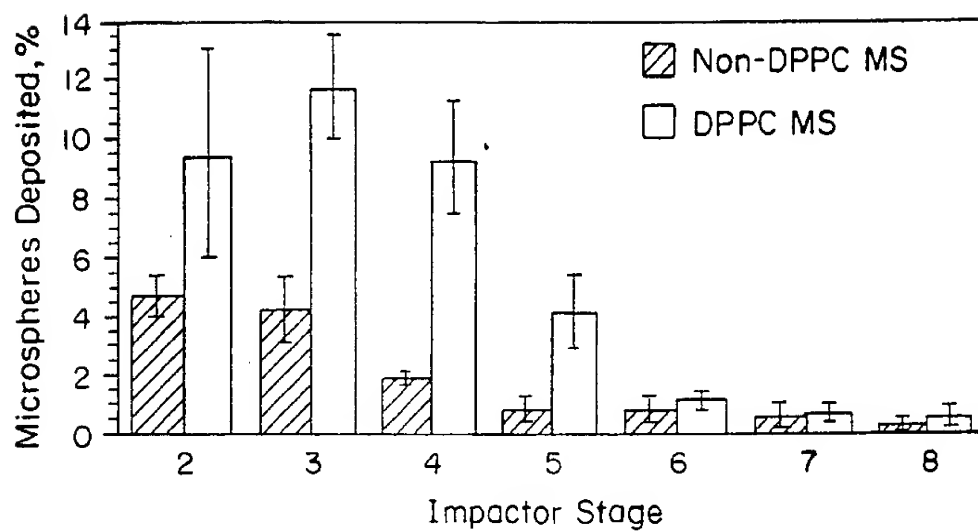


FIG. 2

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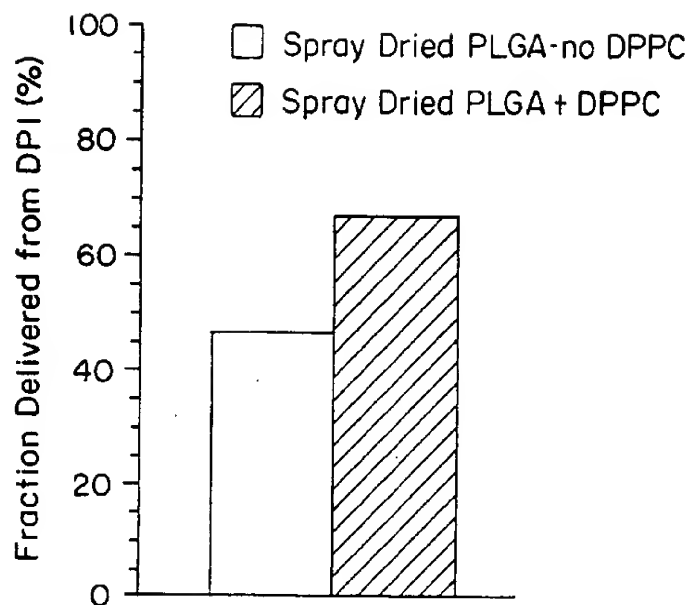


FIG. 3

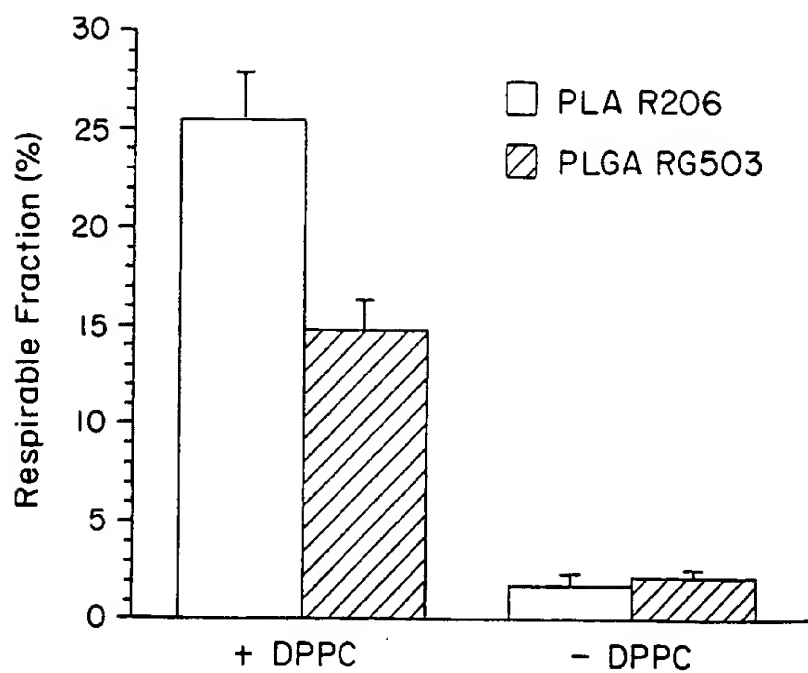


FIG. 4

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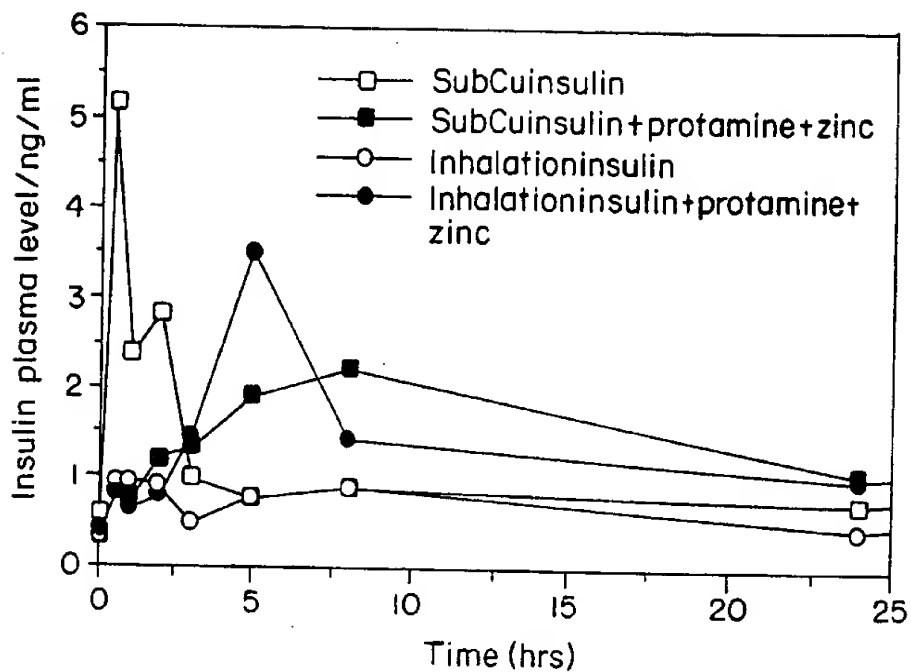


FIG. 5

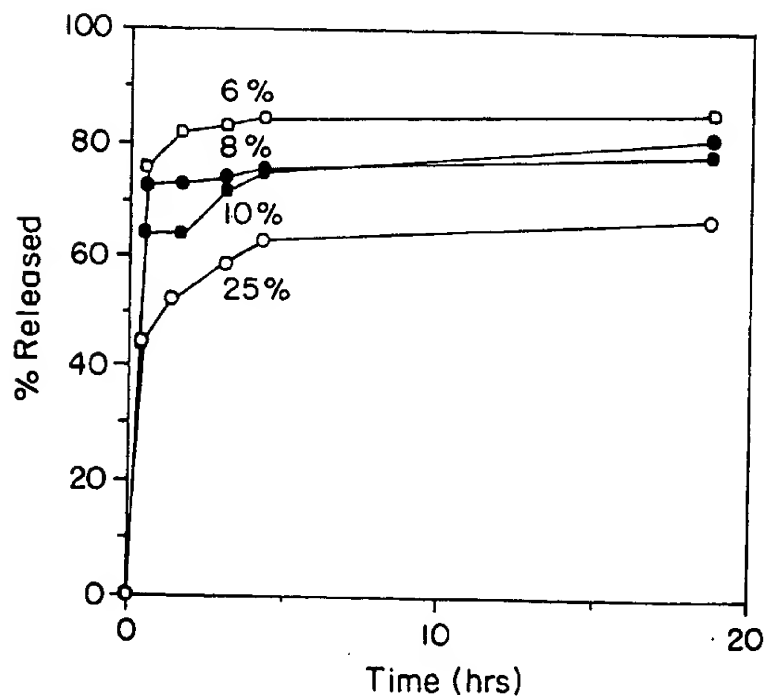


FIG. 6

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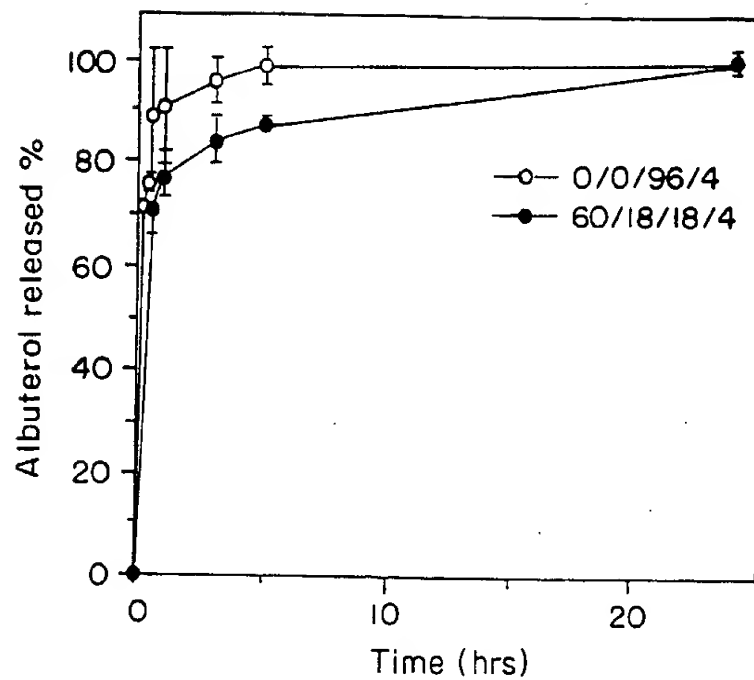


FIG. 7

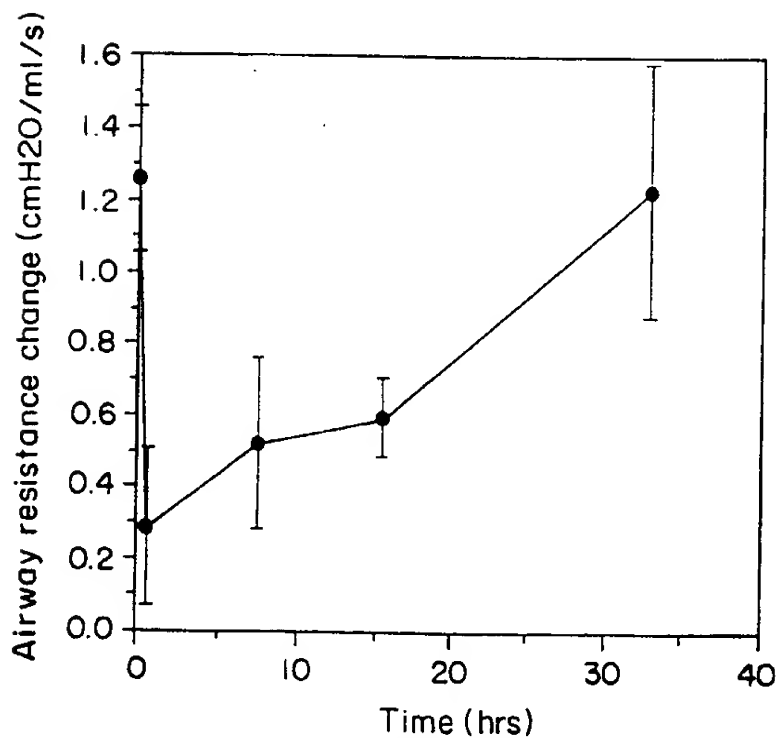


FIG. 8

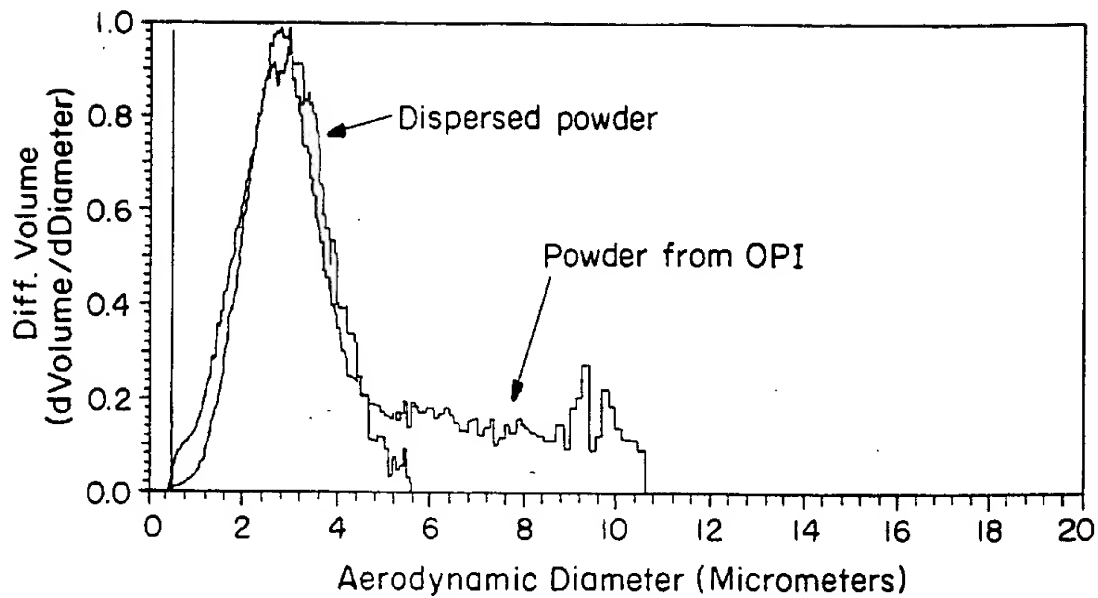


FIG. 9

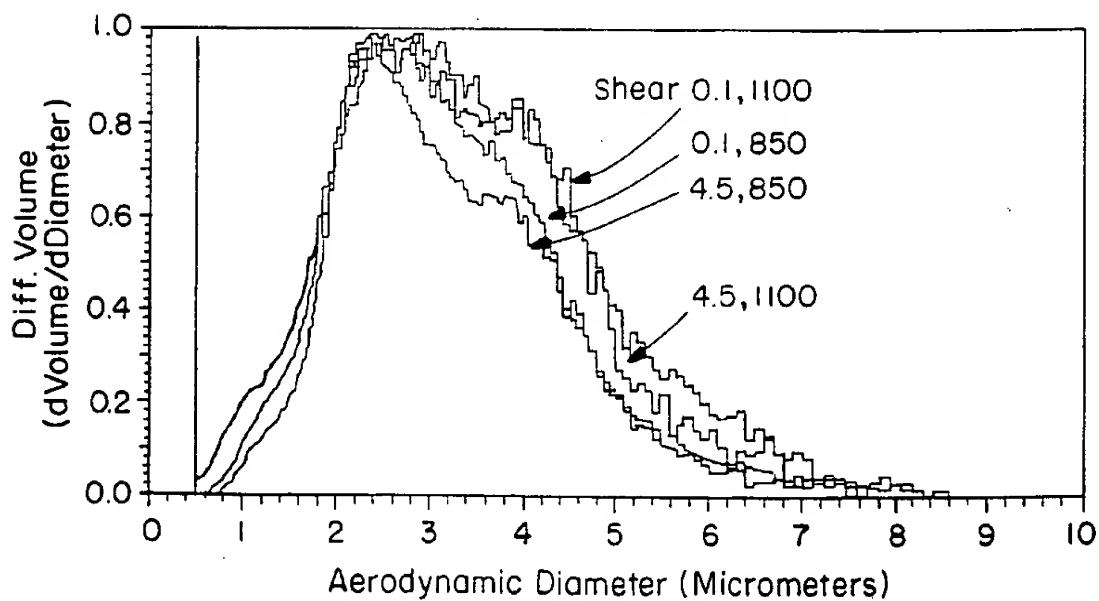


FIG. 10

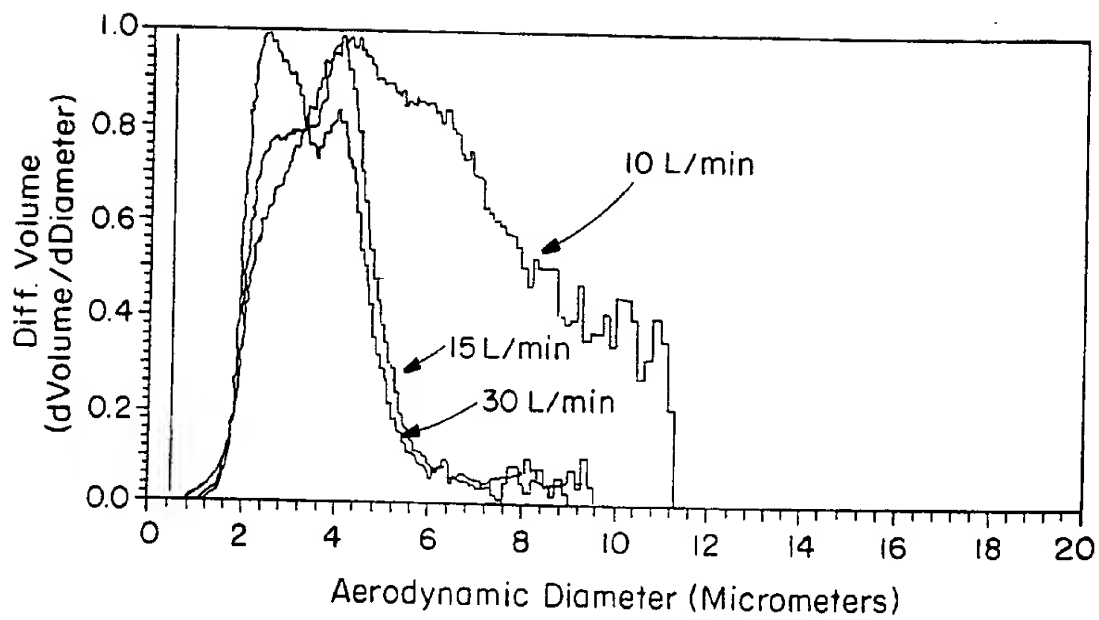


FIG. II

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/14074

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 98 31346 A (MASSACHUSETTS INST TECHNOLOGY ; PENN STATE RES FOUND (US)) 23 July 1998 (1998-07-23) the whole document	1-18
X	HANES J ET AL: "POROUS DRY-POWDER PLGA MICROSPHERES COATED WITH LUNG SURFACTANT FOR SYSTEMIC INSULIN DELIVERY VIA THE LUNG" PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM ON CONTROLLED RELEASE OF BIOACTIVE MATERIALS, US, DEERFIELD, IL., CONTROLLED RELEASE SOC, vol. SYMP. 24, page 57-58 XP002061402 ISSN: 1022-0178 the whole document	1,4-6,8, 9,13-16, 18

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

29 November 1999

Date of mailing of the international search report

15/12/1999

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Authorized officer

Boulois, D

INTERNATIONAL SEARCH REPORT

Inter. nat. Application No

PCT/US 99/14074

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 306 483 A (MAUTONE ALAN J) 26 April 1994 (1994-04-26) column 7 -column 8; example 1 ----	1,2,4-6, 8,9,11, 13-16,18
X	WO 96 09814 A (ANDARIS LTD) 4 April 1996 (1996-04-04) cited in the application page 1, line 24 - line 34 page 5, line 25 -page 6, line 19 page 17; example 3 page 21 -page 23; examples 8,9 ----	8
A	WO 97 44013 A (MASSACHUSETTS INST TECHNOLOGY ;PENN STATE RES FOUND (US); EDWARDS) 27 November 1997 (1997-11-27) claims ----	1-18
P,X	BEN-JEBRIA, ABDELLAZIZ ET AL: "Large porous particles for sustained protection from carbachol-induced bronchoconstriction in guinea pigs" PHARM. RES. (1999), 16(4), 555-561 ,1999, XP002124230 page 555 -page 556 ----	1,2,4-6, 8,9,11, 13-16,18
X	EDWARDS, DAVID A. ET AL: "Large porous aerosols for pulmonary drug delivery" PROC. INT. SYMP. CONTROLLED RELEASE BIOACT. MATER. (1997), 24TH, 67-68 ,1997, XP002124231 the whole document -----	1,6,16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/14074

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-7 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Int. Application No

PCT/US 99/14074

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9831346 A	23-07-1998	US 5855913 A EP 0954282 A	05-01-1999 10-11-1999
US 5306483 A	26-04-1994	US 5174988 A EP 0607118 A WO 9101765 A	29-12-1992 27-07-1994 21-02-1991
WO 9609814 A	04-04-1996	AU 701440 B AU 3530295 A BR 9509171 A CA 2199954 A CZ 9700924 A EP 0783298 A FI 971332 A HU 77373 A JP 10506406 T NO 971438 A NZ 292980 A PL 319600 A ZA 9508239 A	28-01-1999 19-04-1996 16-09-1997 04-04-1996 13-08-1997 16-07-1997 01-04-1997 30-03-1998 23-06-1998 26-03-1997 25-02-1999 18-08-1997 30-09-1996
WO 9744013 A	27-11-1997	US 5874064 A EP 0907356 A	23-02-1999 14-04-1999